Compatibility of biocontrol agent formulas and synthetic fungicides in controlling maydis leaf blight on corn caused by *Bipolaris maydis*

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Abstract. Maydis leaf blight caused by Bipolaris maydis is one of the limiting factors in increasing maize production. The application of biological control agents (BCAs) as a biopesticide to control pests and plant diseases expected to decrease synthetic fungicide usage and its impacts. Several researches showed that the formula of Bacillus subtilis biopesticide suppress the development of several plant diseases including maydis leaf blight in maize. Nevertheless, biological control agents cannot completely replace the need for chemical fungicides in the agro-ecosystem. Therefore, this study was carried out to evaluate the effect and compatibility of several synthetic fungicides with antagonistic bacterial formulas in controlling *B. maydis* on maize. Five types of synthetic active ingredients used were difenoconazole, propinep, fluopicolide, metalaxyl, and dimethomorph. From the results of laboratory tests, 3 types of active ingredients with the highest inhibitory effectiveness be selected for the field test. Field treatments were arranged using a Randomized Block Design with 2 factors, factor I was biological control agents with 2 levels (T₀ = without biological control agents, T₁ = with biological control agents) and factor II was synthetic fungicide with different active ingredients with 4 levels (S₀ = no synthetic fungicide, S₁ = difenoconazole, S₂ = propinep, S₃ = fluopicolide). Each treatment combination was repeated 4 times. The result showed that all the synthetic fungicides tested were compatible with the biological control agent. Fungicide with active ingredient of difenoconazole was the most compatible with the BCAs showing the lowest disease severity value of 27%, while control treatment conditions without BCAs was 72%.

1. Introduction

Plant diseases are still one of the limiting factors that hinder the maize production escalation. Furthermore, these organisms have impact on food security and the availability of sustainable raw materials in their use as bioindustry. There are several diseases caused by fungus pathogen such as maydis leaf blight caused by Bipolaris maydis, banded leaf and sheath blight caused by Rhizoctonia solani, and stem/cob rot caused by Fusarium moniliforme [1].

Biological control takes public attention as an approach to control pathogens because of several issues such as ecology, economic efficiency, and health to support sustainable agriculture [2]. The application of biological control agents (BCAs) as pest and disease control can be formulated in biopesticide products. The targets of using biopesticide technology are: 1) increasing the crop production, 2) increasing income and welfare, 3) controlling plant damage and severity caused by pests and diseases into under economic threshold and 4) reducing the risk of environmental pollution due to excessive use of synthetic chemical pesticides [3].
The application of BCAs to control plant disease is part of the integrated disease control strategies with the combination of biological, physical, and chemical control holistically. In recent years, the formulations of the bacterial antagonist Bacillus subtilis have been produced in the Plant Pathology Laboratory of Indonesian Cereals Research Institute (ICERI). In several publications, B. subtilis is known as an effective agent in controlling major diseases in maize such as downy mildew [4], leaf blight, and midrib rot [5]; [6];[7].

Besides the ability of the antagonistic bacteria of B. subtilis to suppress the development of R. solani in vitro, it also increased the germination and vigor of corn seeds. [8] and [9] found that the antagonistic bacterial B. subtilis TM4 did not have negative affect on seed germination but on the contrary, increasing plant growth and yield. ICERI has produced a biopesticide with an active consortium of B. subtilis bacteria called Tribas which has been patented (No. IDP000057068). Limited scale efficacy tests (laboratory/greenhouse) and extensive trials have been carried out to confirm that product can control the plant diseases [7]; [8].

In order to see the performance development of Tribas, a wide scale of the Tribas has been carried out in the farmer level by applying Tribas to the seed production of 5 elite elders of a number of hybrid maize varieties of IAARD. The test results showed that the Tribas biopesticide applied to the five lines through seed treatment and crop spraying was relatively able to inhibit the development of disease infections in plants and increase the growth of the plants tested compare to lines without treted with Tribas [10].

The purpose of the study was to determine the compatibility between the formula of BCAs and several synthetic fungicides on the severity of maydis leaf blight on maize.

2. Materials and Methods
The research was carried out at the Plant Pathology Laboratory and Bajeng Food Crops Research and Assessment Installation, Indonesian Cereals Research Institute from August to December 2020.

2.1. Laboratory test
Five types of synthetic materials were investigated using a Completely Randomized Design with 5 replications. Then, 3 types of synthetic active ingredients which had the highest inhibitory effectiveness were selected for field assessment.

| No. | Active ingredients | Dosage of recommendation |
|-----|--------------------|--------------------------|
| 1   | Diphenocnoazole 150 g/l & propiconazole | 3 – 4,5 ml/l |
| 2   | Propinep 70%       | 2 kg/ha                  |
| 3   | Fluopicolide 6%    | 2 kg/ha                  |
| 4   | Metalaxyl 35%      | 1.25 g/kg seeds          |
| 5   | Dimetomorph 500 g/l & pyraclostrobin 10 g/l | 5 ml/kg seeds |

2.1.1. Inoculum preparation of B. maydis fungi
Isolation of leaf blight symptoms was carried on by cutting the symptomatic corn leaves into pieces with a size of 0.3 x 0.3 cm. The pieces were sterilized using distilled water for 2 minutes, then soaked into 70% alcohol for a minute, rinsed again with distilled water for a time and dried it on sterile filter paper. The dried leaves were incubated in growing media, Potato Dextrose Agar (PDA), and incubated at room temperature. Purification was performed immediately after the hyphae grew 2-3 days after isolation. Pure isolates of B. maydis were identified to confirm the species microscopically by observing the shape of conidia and hyphae.
2.1.2. Preparation of biocontrol agent formula
The biocontrol agent’s formula used in this study was a collection of Indonesian Cereals Research Institute. The bacterial isolates were rejuvenated on Nutrient Agar (NA) growing media and incubated at room temperature for 48 hours. The production of biocontrol agent’s formula was carried out in a laminar air flow cabinet.

2.1.3. Compatibility test of biocontrol agent’s formula and synthetic materials
The compatibility of the BCA’s formula was tested using the disk diffusion method. The BCAs were grown on Nutrient Broth (NB) liquid media in the form of a suspension. Suspension was prepared by growing the BCA’s formula into 10 mL NB and incubating on a shaker at 100 rpm for 24 hours. Compatibility test was carried out by dripping 10 L of suspension of synthetic active ingredients on sterile filter paper with a diameter of 5 mm contained in NA media which had been spread with 100 L of BCA’s suspension. Pairs of bacterial isolates were compatible if there was no clear zone formed after testing with BCA and synthetic positions vice versa.

2.1.4. Inhibition efficiency of synthetic active ingredients on B. maydis
As many as 100 µl of each synthetic active ingredient based on the application dose (Table 1) was added to the PDA before solidifying and mixed evenly. Mycelium of B. maydis fungus was cut (5 mm in diameter) and aseptically placed in the center of each petri containing the toxic medium. The treatment without synthetic active ingredient was made as a control under the same conditions. For each treatment, five replications were made. Petri dishes were incubated at 27°C and the diameter of the fungus colonies was measured 5 days after inoculation.

The inhibition efficiency (IE) of synthetic active ingredients on the growth of fungal isolates was calculated based on the formula:

\[
IE (%) = \left(\frac{\text{diameter of control} - \text{diameter of treatment}}{\text{diameter of control}}\right) \times 100
\]

2.2. Field Test
The treatments were arranged using a randomized block design with 2 factors, factor I (BCAs formula) with 2 levels (T0 = without BCA, T1 = with BCA); factor II (synthetic fungicide with different active ingredients) with 4 levels (S0 = no synthetic fungicide, S1= diphoneconazole S2= propinep S3= fluopicolide). Each combination treatment was repeated 4 times.

2.2.1. Seed Treatment
Two hours before planting, 100 g of corn hybrid seed of T1 level was given seed treatment by mixing 8 g of BCA’s formula. After that, the seeds were planted in experimental plots of 3 x 5 m with a spacing of 75 x 25 cm. Fertilization was carried out at 2 and 4 weeks after planting (WAP) using urea 300 kg and NPK (15:15:15 w/w/w) 200 kg per hectare.

2.2.2. Inoculation of pathogenic B. maydis
Inoculation of plants with B. maydis cultured in PDA media was conducted by spraying the suspension of pathogenic culture of 106 CFU/ml conidia density on maize leaves at 3 weeks after planting (WAP)

2.2.3. Application of BCA’s formula and synthetic active ingredients
The applications of the BCA’s formula were at 15, 25, and 35 days after planting (DAP). The application was carried out by dissolving 3 g/l of the formula into water, then spraying it evenly on the plant surface. The application of synthetic fungicides was carried out at 45 DAP according to the dose of each fungicide used.

Observation variables included the intensity of leaf blight (30, 45, 60, 75 DAP), plant height (30 and 60 DAP), and crop yields. The data obtained were then analyzed using GLM procedure (General Linear
Model) for the linear regression model and Duncan’s new multiple range test (DMRT) for the multiple comparison with significance 95%. All the data was analyzed by STAR 2.0.1 software [12].

Table 2. Severity of leaf blight scale [13]:

| Scale | Description |
|-------|-------------|
| 0     | No symptoms of disease |
| 1     | Very few infections, visible one, two, or few lesions on the lower leaves |
| 2     | Mild infection, moderate number of lesions on lower leaves only |
| 3     | Moderate infection, abundant lesions on lower leaves, few on middle leaves |
| 4     | Severe infection, abundant lesions on lower and middle leaves, leading to upper leaves |
| 5     | High infection, abundant lesions on all leaves, plants become dry or even die |

The scale observation was transformed to the following percentage of infection formula:

\[ I (%) = \frac{\sum (n \times v)}{Z \times N} \times 100\% \]

I : intensity of disease  
\( n \) : number of infected plants in each category  
\( v \) : scale value for each infected plant  
\( Z \) : the highest scale value  
\( N \) : number plants observed

3. Results and Discussion

3.1. Laboratory Test

All synthetic fungicides tested caused pathogen inhibition (Table 2) which tended to be very significant in the growth (0.44-37.45%) of \( B. \) \textit{maydis} at 3 days after isolation (DAI). Diphenonozole was found to be the most effective in reducing the growth of fungi compared to that of other synthetic fungicides followed by propinep, fluopicolide, pyraclostrobin, and metalaxyl. In the 3 DAI observations, the diphenonozole treatment had the highest inhibitory effectiveness of 37.45% and was not significantly different from the propinep treatment (24%) but significantly different from the other treatments (0-23%).

The results of statistical analysis at 5 DAI showed that synthetic fungicide treatment had a significant effect on the effectiveness of inhibiting the mycelia growth of the fungus \( B. \) \textit{maydis}. It was observed that diphenonozole was quite toxic to fungi by inhibiting its growth until day 5 (44%) that significantly higher than the metalaxyl treatment (13%) and control (0%). This result was in agreement with [14] study in which the application of a fungicide with the active ingredient of probenazole was an effective way to control corn leaf blight from the early to late stages of plant growth.
Table 3. The effectiveness of synthetic fungicide inhibition on the growth of mycelia diameter of *B. maydis* observed at 3 and 5 DAI

| Fungicide active ingredients | 3 DAI   | 5 DAI   |
|------------------------------|---------|---------|
| Control                      | 0.00 c  | 0.00 c  |
| Diphenoconazole              | 37.45 a | 44.93 a |
| *Propinep*                   | 24.59 ab| 31.72 ab|
| *Fluopicolide*               | 23.15 b | 33.61 a |
| *Metalaxyl*                  | 0.44 c  | 13.08 bc|
| *Dimetomorph*                | 19.89 b | 30.35 ab|

Numbers followed by the same letter in the same column are not significantly different in the DMRT test = 0.05

The inhibition of *B. maydis* growth was observed to vary between treatments (Figure 1). The active ingredients of synthetic fungicides of *fluopicolide* and *propinep* were observed to provide equal inhibition, while BCA’s treatment suppressed the growth of *B. maydis* by forming a clear zone around the fungal colonies. It was suspected that *B. maydis* could not synthesize the compounds the fungi needed and had to obtain them from the treated PDA media and finally inhibited the growth of fungal mycelia. [15] stated that all fungi required almost the same essential elements but had different abilities to obtain them from growth media.

![Figure 1. B. maydis on media treated with pesticide on 5 DAI](image)

Note: Image from left to right: *fluopicolide, propinep, BCA, no pesticide*

Compatibility testing between BCA and synthetic fungicides showed that the active ingredient *diphenoconazole* was compatible with BCA growth characterized by the absence of a clear zone around the filter paper (Figure 2). Other than that, *fluopicolide* was observed compatible but *propinep* was less compatible with BCA based on the formation of a clear zone.

Integrated control using biological, vegetable and chemical agents is recommended as an effort to suppress the development of plant diseases in the field. Based on [16] the combination of *Pseudomonas fluorescens + Trichoderma viridii + Bacillus subtilis* 16 followed by the combined application of neem + manure + zinc sulfate was found to be significantly superior in reducing the incidence of neck and root rot disease of *Lasiodiplodia theobromae* in peanuts.
Figure 2. Testing the synergy between BCA and the active ingredients of synthetic fungicides, from left to right: *diphenoconazole* (-), *fluopicolide* (-), and *propinep* (+) on media treated with BCA formula after 5 days of incubation.

Note: (+) clear zone (incompatible)
(-) unclear zone (compatible)

3.2. Field Test

The percentage of the severity of maydis leaf blight on maize treated with BCA and synthetic fungicides increased in each observation (Table 3). The application of BCA significantly affected the rate of disease infection, at 30 DAP all treatments were observed to be infected with maydis leaf blight. The lowest disease severity was found in the treatment with BCA + *diphenoconazole* (22%) that was significantly lower with the treatment without BCA, single BCA treatment and BCA + *propinep* treatment respectively.

After 45 DAP, treatment with BCA had a significant effect on the severity of leaf blight. Treatment with BCA + *diphenoconazole* observed an infection rate of 23.5%, significantly lower than all treatments without BCA (43-59%). Similarly, at 60 DAP, the treatment without BCA (49-59%) was observed to have significantly higher disease severity than the treatment with single BCA (control) by 31%, BCA + *diphenoconazole*, and BCA + *propinep* (25 and 33%). Apparently, seed treatment using BCA prior to pathogen infection gave positive efficacy. The preventive effect of Iturin A2 as an antifungal agent produced by antagonistic bacteria of *B. subtilis* was superior to the control effect, so it was recommended that it was applied before or at the onset of the disease to obtain effective control of maydis leaf blight [17].

At 75 DAP, the application of BCA as seed treatment and spraying on plants showed have a significant effect on leaf blight infection. The intensity of maydis leaf blight disease with BCA treatment classified as moderate respectively was BCA + *diphenoconazole* (27%), single BCA/control (32%), BCA + *propinep* (35%). The disease severity without BCA was significantly higher (50-72%) than with BCA (27-48%).

The application of synthetic fungicides was observed having no negative impact on the mechanism action of BCA. Maydis leaf blight infection was found lower in the BCA combine with synthetic fungicides treatment than in the single synthetic fungicide without BCA. In line with the research results of [18], *Trichoderma* could be applied in the field in combination with several chemical fungicides at low concentrations, which could increase biocontrol efficiency and contribute to environmentally friendly pathogen control.
Table 4. The average disease severity (%) of maydis leaf blight in the treatment of various types of pesticides at 30, 45, 60, and 75 DAP

| Treatment         | Intensity of disease (%) DAP |
|-------------------|------------------------------|
|                   | 30  | 45  | 60  | 75  |
| No BCA Control    | 53.5 f | 59.0 e | 59.5 d | 72.5 e |
| Diphenoxonazole   | 38.0 cde | 43.0 d | 49.5 c | 50.5 c |
| Propinep          | 45.5 def | 47.5 d | 56.5 d | 66.5 d |
| Fluopicolide      | 46.5 ef | 48.0 d | 53.5 cd | 53.5 c |
| BCA Control       | 27.0 ab | 29.0 ab | 31.0 ab | 32.5 ab |
| Diphenoxonazole   | 22.0 a  | 23.5 a  | 25.0 a  | 27.0 a  |
| Propinep          | 30.5 abc | 31.0 bc | 33.0 b  | 35.0 b  |
| Fluopicolide      | 35.5 abc | 35.5 c  | 47.5 c  | 48.0 c  |

Note: numbers followed by the same letter in the same column are not significantly different in the DMRT test =0.05

BCA treatment and synthetic fungicide applied to maize plants had a significant effect on plant height (Table 4). The results of statistical analysis were observed at 30 DAP treatment with BCA + diphenoxonazole (162 cm) significantly higher than the treatment without BCA (142-152 cm) or with BCA + fluopicolide and single BCA treatment (control) (157 and 155 cm), but not significantly different from the BCA + propinep treatment (160 cm). Observations at 60 DAP showed that the BCA + diphenoxonazole (227 cm) treatment was not significantly different from the control treatment (single BCA) (223 cm), but significantly different from the other treatments (209-219 cm). The results of plant height in this study were thought to affect the production of maize. [19] mentioned that plant height was an important morphological trait that affects maize crop yields.

Table 5. Average plant height (cm) at 30 and 60 DAP from the treatment of various types of pesticides on maize plants inoculated with B. maydis

| Treatment            | Plant height (cm) |
|----------------------|-------------------|
|                      | 30 DAP            | 60 DAP            |
| Control              | 1429 f            | 209.0 d           |
| Diphenoxonazole      | 152.5 de          | 213.8 cd          |
| Propinep             | 148.6 e           | 211.3 d           |
| Fluopicolide         | 150.4 e           | 214.6 cd          |
| BCA                  | 155.3 cd          | 223.8 ab          |
| BCA + Diphenoxonazole| 162.4 a           | 227.0 a           |
| BCA + Propinep       | 160.8 ab          | 219.4 bc          |
| BCA + Fluopicolide   | 157.4 bc          | 218.0 bc          |

Note: numbers followed by the same letter in the same column are not significantly different in the DMRT test =0.05

The results showed that the application of BCA and synthetic fungicides had a significant effect on the observation of crop yields (Figure 3). Treatment with single BCA (control) (9.9 tons/ha) was observed to be not significantly different from the BCA + diphenoxonazole treatment (10.8 tons/ha) but significantly different from other treatments (8.1-8.8 tons/ha). The decrease in yield was thought to be due to the loss of active leaf area for the photosynthesis process. The higher the lesion on the leaf, the more photosynthetic area destroyed by the pathogen causing maximum yield loss [20]. Meanwhile, [21] reported that the fungicide Zineb 75 WP 0.25% was found to be most effective in inhibiting the development of leaf blight, lower disease severity and ultimately resulted in higher maize grain yields.
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Figure 3. Average yield (tonnes/ha) from the treatment of various types of pesticides on maize inoculated with *B. maydis*

Note. T0S0 = control; T0S1 = *diphenaconazole*; T0S2 = *propinep*; T0S3 = *fluopicolide*; T1S0 = biological control agent; T1S1 = biological control + *diphenaconazole*; T1S2 = biological control + *propinep*; T1S3 = biological control + *fluopicolide*.

The mean values followed by different letters mean significantly different from each other according to Duncan's test at p = 0.05. The bar shows the average standard error value based on four replications.

4. Conclusion

There was a positive synergy between biological control agent and synthetic fungicides (active ingredient diphenaconazole) in controlling *B. maydis* leaf blight on maize. So it is suggested for the best control method for maydis leaf blight. The biological control treatment combined with the application of a synthetic fungicide with the active ingredient of *diphenaconazole* had the lowest disease severity up to 27% in the control treatment condition without biological control at 72%. The application of synthetic fungicides did not negatively affect the performance of biological control in controlling leaf blight in maize.

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