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Abstract. Downy mildew caused by Peronosclerospora spp. is a major limiting factor in maize production in Indonesia. Susceptible varieties can have yield losses of around 90 to 100%. The continuous use of metalaxyl has triggered resistance to downy mildew. Therefore, it is necessary to have other fungicides that can replace the use of metalaxyl. In this study, the oxathiapiproline-famoxadone was tested in combination with the fungus Trichoderma sp. as an alternative to metalaxyl. This study used a randomized complete block design. The treatments were arranged in a factorial with 2 factors. The first factor was fungicide (without/F0 and with fungicide/F1). The second factor was Trichoderma sp., i.e. T0 (without Trichoderma sp), T1 (Trichoderma sp. 106 spores/ml), T2 (Trichoderma sp. 107 spores/ ml), and T3 (Trichoderma sp. 108 spores/ml). The results showed that oxathiapiproline-famoxadone was able to suppress downy mildew, while the application of Trichoderma sp. did not affect the control of downy mildew and maize plant growth. Fungicide application and Trichoderma sp. 107 spores/ml suppressed disease severity at 7 days after inoculation with Peronosclerospora sp.
Keywords: Oxathiapiproline-famoxadone fungicide, Trichoderma sp, Maize

1. Introduction

Maize (Zea mays L.) is one of the important crops in Indonesia. [1] stated that maize is a strategic commodity in Indonesian agricultural and economic development due to its multipurpose function not only for food but also for industry. However, downy mildews have been reported to be one of the major devastating diseases attacking maize plants in many countries [2], [3] including in Indonesia.

Peronosclerospora and Sclerospora species cause downy mildew in maize crops in tropical Asian countries [3]. These species were also reported to cause downy mildew in sorghum in India and Thailand [4]. Maize downy mildew in Lampung, one of the provinces in Indonesia, has been reported by [5] caused by P. sorghi, P. maydis, and P. philippinensis.

The control of this destructive disease is usually through the treatment of seeds with certain fungicides prior to planting. One of the most popular fungicides used in controlling maize downy mildew as seed treatment is metalaxyl. However, the continuous use of metalaxyl without a change with other fungicides has triggered resistance to downy mildew [6], [7]. Damage to the conidia of Peronosclesrospora sp. exposed on the metalaxyl was lower than to those treated with fenamidone and dimetomorph [8]. The low conidial damage indicates a decrease in the inhibitory activity of the metalaxyl against Peronosclerospora sp.

Thus, it is necessary to use other fungicides that can replace the application of metalaxyl. In this study, oxathiapiprolin-famoxadone was tested as an alternative to metalaxyl. Oxathiapiprolin acts

systemically and according to [9] oxathiapiprolin can control plant pathogenic oomycetes. Famoxadone is a strobilurin group fungicide. This class of fungicides has a lower rate of toxicity to non-target organisms and is an environmentally friendly fungicide [10]

Researchers are also looking for the solution to suppress the negative effect of downy mildew on plant production using biological control agent due to their environmental safety and ability to induce plant disease resistance to pathogens. Trichoderma spp. are frequently utilized as biological control agents against various plant diseases. Several mechanisms are employed by Trichoderma spp. against fungal pathogens include antibiosis by which they produce secondary metabolites such as peptaibols, terpenes, polyketides, gliotoxin, and gliovirin. Other mechanisms are by mycoparasitism, competition for nutrients and space with the pathogens, the promotion of plant growth and development, the induction of plant defense mechanisms as well as changing the conditions of the environment [11]. Trichoderma sp. enter the plant tissue through the roots and plants and provide an induced resistance signals for phytoalexin activation, one of which is salicylic acid. Salicylic acid provides an important signal in the resistance mechanism to inhibit microorganisms and causes plant tissue to become more resistant to disease [11]. In this study, a combination of oxathiapripoline-famoxadone and Trichoderma sp. will be tested to control downy mildew and increase the growth of maize plants.

2. Materials and Method

This research was conducted at the Plant Disease Laboratory, Department of Plant Protection and Integrated Field Laboratory, Faculty of Agriculture, University of Lampung, in July-October 2020. This study used a randomized block design. The treatments were factorially arranged with 2 factors. The first factor was fungicide (F0 = without fungicide and F1 = with fungicide). The second factor was Trichoderma sp. (T0 = without Trichoderma sp., T1 = density 106 spores/ml, T2 = density 107 spores/ml, and T3 = density 108 spores/ml). This study consisted of 8 treatments and 3 replications, in order to obtain 24 experimental units. One experimental unit consisted of 10 plants planted in one polybag.

The materials used were maize seed P-27, fungicide with active ingredient of oxathiapiprolin-famoxadone, sterile soil, fungus isolate Trichoderma sp, and inoculum of downy mildew. The seeds and the fungicide were obtained from the market. The Trichoderma sp. isolate was isolated from healthy maize roots among the diseased plant using potato sucrose agar. Soil was mixed with manure (2:1), sterilized in a drum at 100oC and placed in a polybag in which the seeds were grown. The downy mildew inoculum was prepared from maize leaves with heavy symptoms. Spore suspension (500µl) with spore density of 105 spores per ml of water was inoculated at plant growing point of 6 days old plants and the spore suspension was inoculated at 4.00 am. Prior to inoculation, the water contained in the growing point of the plant was removed.

The observation variables were incubation period, disease incidence, disease severity, plant height, number of leaves, as well as shoot and root dry weights. The data were analyzed by analysis of variance, and the differences in mean value between treatments were tested with the Least Significant Difference (LSD) tests at the 5% level. The incidence of disease is calculated using the following modified formula of [5]:

$$I = n / N \text{ X } 100\%$$

where, I = disease incidence (%), n = number of plants shown symptoms, and N= the number of all plants observed. Disease severity was calculated based on scoring of disease: 0 = no symptoms observed, 1 = when <10% of leaf surface showed the symptoms, 2 = when 10-25% showed the symptoms 3 = when 25-50% showed the symptoms and 4 = when more than 50% of leaf surface showed the symptoms. The scoring data were calculated using the following formula:

$$S = (\sum (nxv)) / (NxV) \text{ X } 100\%$$

where, S = severity of disease (%), n = number of leaves with a certain score, v= the score of each category of attack, N = number of leaves observed (sample), V = value of the highest score of disease.

3. Results and discussion

Our results showed Oxathiapiprolin-famoxadone could control downy mildew. This is evidenced by the decreased incidence of downy mildew, reduced disease severity, and an extended incubation period. The application of Trichoderma sp. in single treatment did not affect the control of downy mildew and growth of the maize plants. In this study, fungicide application also affected maize growth, i.e. plant height and number of leaves.

3.1. Effect of fungicide and Trichoderma sp. on period of incubation

The incubation period is the time between inoculation and when the first symptoms appear on plants. Application of oxathiapiprolin-famoxadone caused a longer incubation period than the treatment without fungicides. The incubation period on plant treated with fungicides was 10 days after inoculation (DAI) and 7 DAI on non-fungicides (Table 1). Oxathiapiprolin was reported acts at a very low concentration and at multiple stages of asexual pathogen life cycle. The preventative mechanism is by inhibiting release of zoospore, affecting zoospore motility and germination of sporangia. When used curatively, oxathiapiprolin stops growth of mycelium inside of the host plant before lesions occur and protects plants one to two days post inoculation [9].

Table 1. Effect of oxathiapiprolin–famoxadone fungicide on incubation period of maize downy mildew

Treatments	Incubation period (DAI)
Fungicide	
F0	7.69 b
F1	10.50 a
LSD	1.77

Note. Numbers followed by different letter are significant according to LSD (<5%)
F0=without fungicide, F1=with fungicide, DAI = days after inoculation

3.2. Disease incidence

Oxathiapiprolin–famoxadone application significantly reduced disease incidence (Table 2). The percentage disease incidence by fungicide application at 28 days after inoculation was significantly lower (7.70%) compared to without fungicide (9.72%).

Table 2. Effect of oxathiapiprolin–famoxadone fungicide on maize downy mildew disease incidence

Treatments	Incidence of downy mildew (%)	
	28 DAI	35 DAI
Fungicide		
F0	9.72 a	9.72 a
F1	7.70 b	7.70 b
LSD	0.83	0.83

Note. Numbers followed by different letter are significant according to LSD (<5%) F0=without fungicide, F1=with fungicide, DAI = days after inoculation

At 28 and 35 DAI, there was no difference in the incidence of downy mildew. It is assumed that after 28 days, no increase in the incidence of downy mildew caused by Peronosclerospora sp. occurred [12] as older plants are more resistant to this disease. Maize plants are susceptible to infection with downy mildew at an early stage until the formation of second and third leaves, after which susceptibility decreases.

The effect of treatment on the number of rice sapouts showed similar results (Fig. 2). The use of biochar 30 tons ha-1 mixed evenly with soil accompanied by the use of BiO2 provides a number of saplings that tend to increase. The number of sapouts is higher than the control and treatment of NPK.

3.3. Disease Severity

The results showed that the fungicide application had a significant effect on maize downy mildew severity, but not in Trichoderma sp. There was an interaction between the fungicide and Trichoderma sp. on the severity of downy mildew at 7 days after inoculation.

The combination treatment of oxathiapiprolin–famoxadone and Trichoderma sp. showed that the lowest severity level was in the F1T2 treatment at 7 days after inoculation with Peronosclerospora sp., while the control treatment had the highest disease severity (Table 3). This is presumably due to a positive interaction between fungicides and Trichoderma sp. Trichoderma sp. are able to reduce the severity of downy mildew, as they have the ability to increase defense enzymes in plants such as peroxidase. According to [13], this enzyme strengthens plant cell walls against the degradation by enzymes produced by pathogens through the formation of structural proteins. Strengthening the cell wall causes pathogens to be unable to penetrate into plant leaf cells. In addition, the oxathiapiprolin is a systemic fungicide that works by inhibiting the growth of disease in host plants and suppresses the production of spores lead to reduce the growth of pathogens [6].

Table 3. Effect of oxathiapiprolin–famoxadone and Trichoderma sp. combination on the severity of maize downy mildew at 7 days after inoculation

Treatments	Severity (%)
F0T0	19.13 a
F0T1	6.89 cd
F0T2	14.75 ab
F0T3	12.27 bc
F1T0	3.52 d
F1T1	4.58 d
F1T2	3.52 d
F1T3	4.13 d
LSD	6.32

Note. Numbers followed by different letter are significant according to LSD (<5%) F0=without fungicide, F1=with fungicide, T0= without Trichoderma sp., T1=Trichoderma sp (106 spores/ml), T2= Trichoderma sp (107 spores/ml), T3=Trichoderma sp (108 spores/ml).

3.4. Plant Height

Analysis of variance showed that compared to non-fungicide treated plants, the plants with fungicide were significantly different in plant height, while the application with Trichoderma sp. had no significant effect on height of plant. The results also showed the interaction between the fungicide and Trichoderma sp. had no significant effect on plant height. LSD tests showed the fungicide application had a significant effect at 28, 35, and 42 DAS (day after sowing). The data show that the plant height in the fungicide application is greater than no fungicide treated plant (Table 4).

Table 4. Effect of oxathiapiprolin-famoxadone fungicide on maize plant height

Treatments	Plant height (cm)		
	28 DAS	35 DAS	42 DAS
Fungicide			

F0	63.59 b	70.14 b	74.98 b
F1	74.52 a	85.83 a	91.45 a
LSD	8.99	8.82	8.62

Note. Numbers followed by different letter are significant according to LSD (<5%)F0=without fungicide, F1=with fungicide, T0= without Trichoderma sp., T1=Trichoderma sp (106 spores/ml), T2= Trichoderma sp (107 spores/ml), T3=Trichoderma sp (108 spores/ml). DAS = Days after sowing

3.5. Number of leaves

The fungicide application caused significant increase in the number of leaves compare to Trichoderma sp. application (Table 5), and there was no interaction between the fungicide and Trichoderma sp. application in the number of plant leaves.

Table 5. Effect of oxathiapiprolin-famoxadone fungicide on number of maize leaves

Treatments	Number of leaves
	42 DAS
Fungicide	
F0	9.95 b
F1	10.51 a
LSD	0.29

Note. Numbers followed by different letter are significant according to LSD (<5%) F0=without fungicide, F1=with fungicide, DAS = Days after sowing

The results indicate a significant effect of oxathiapiprolin-famoxadone on plant growth. This is thought to be due to indirect effect of fungicide that suppress downy mildew thus plant growth can be maximized. This is indicated by the higher plant height, greater number of leaves, as well as increased shoot and root dry weights compared to those without fungicides. Research by [14] shows that fungicides application has an effect on the intensity of disease in plants. Disease in the leaves can disrupt photosynthetic processes, so that the absorption and formation of nutrients needed by plants can result in disrupted growth. Due to the application of fungicides, the loss of leaf area is smaller. Therefore, the physiological processes such as photosynthesis are not disturbed.

3.6. Shoot and Root Dry Weight

The results showed that fungicide application had a significant effect on shoot and root dry weight, and there was no interaction between the fungicide and Trichoderma sp. application on plant dry weight. The shoot and root dry weights of the plants with fungicide treatment was greater than that without fungicide treatment (Table 6).

Table 6. Effect of oxathiapiprolin-famoxadone fungicide on maize shoot and root dry weight

Treatments	Dry Weight (g)	
	Root	Shoot
Fungicide		
F0	0.89 b	2.46 b
F1	2.59 a	5.29 a
LSD	1.12	1.74

Note. Numbers followed by different letter are significant according to LSD (<5%) F0=without fungicide, F1=with fungicide.

Application of Trichoderma sp. did not have a significant effect on some of the observed variables, presumably due to the environmental influence on the fungal growth or a lack of time required for the fungus to be able to interact with plants, therefore it has not been able to induce resistance. It is suspected that the fungicide reduced the germination ability of the initial spore inoculum, then the germinated spores form and spore in the soil then restore the number of spores in the soil.

4. Conclusion

Previous studies have shown that T. harzianum is insensitive to the mancozeb. Another thing that could affect Trichoderma sp. against downy mildew is the absence of a carrier. According to [14], for optimal growth, fungi need adequate nutrition as an energy source. The energy source for the Trichoderma sp. one of them can be obtained from the carrier media. The nutrients contained in the carrier material are also an important factor that can affect the growth of T. harzianum and also affect its viability (survival).

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