Original Research Article

THE EFFECTIVENESS OF *TRICHODERMA* AS BIOFUNGICIDE AND INDUCER OF CORN PLANT RESISTANCE AGAINST DOWNY MILDEW DISEASE

ABSTRACT

One of environmentally friendly controlling is the use of *Trichoderma* spp. as a natural controlling agent. The objective of this research was to find out the effectiveness of *Trichoderma* spp. as a biofungicide, as an inducer for corn plant resistance, and the combination of both, against downy mildew disease. This research was conducted in the plant pest and disease laboratory in the Agrotechnology Department of Faculty of Agriculture in Lampung University. This research used completely randomized design (CRD) consisting without treatment (0), *Trichoderma* spp. GDR isolate (1) *Trichoderma* spp. NTF isolate (2), and *Trichoderma* spp. TRJ isolate (3) treatments which were applied to the plant growing points as fungicide (B) and as inducer of plant resistance to be applied in the plant roots (P).The research results showed that the *Trichoderma* spp. treatments as biofungicide and inducer of plant resistance could reduce the disease occurrence at 4 and 5 days after inoculation (DAI), but they could not reduce the disease severity and improve stover dry weight of corn plant. The *Trichoderma* spp. treatment as biofungicide and inducer of plant resistance could extend the incubation time and reduce downy mildewdisease significantly at the early course of the disease.

Keywords : biofungicide, downy mildew, plant resistance inducer, *Trichoderma* spp.

1. Introduction

Corn (*Zea mays* L.) is one of harvest crops which has an important economy value in Indonesia. The corn seeds contain of carbohydrate, protein, fat, vitamin, and important minerals. Lampung province is one of corn production center areas in Indonesia, and the areas are distributed in some districts like South Lampung, East Lampung, and Middle Lampung.

According to Central Bureau of Statistic (2016), the dry peeled corn production in Lampung in 2012-2015 decreased from 1,760,275 ton in 2012; 1,760,278 ton in 2013;1,719,386 ton in 2014; into 1,502,800 ton in 2015. Decreasing trend of corn production is related to problems in the existing corn culturing such as pest and disease attacks, and one of important disease in corn plant is downy mildew disease.

The downy mildew disease is caused by *Peronosclerospora* spp. fungi. The potential of corn production cannot be obtained if the corn is infected by the downy mildew disease (Lukam *et.al.,* 2016). This disease in the beginning only occurred in some corn plantation areas in Indonesia, but then it spread into some provinces. This downy mildew reduces up to 90% of

corn production, especially when the pathogen infection occurs at the early of vegetative growth course (Hoerussalam *et.al.,* 2013).

The downy mildew disease can be controlled by using resistant varieties, environment sanitation, plant rotation, simultaneous planting time arrangement, and treatment of seedsby using synthetic fungicide which one of them uses active metalaxyl substance. Continuous use of metalaxyl in long term can cause resistance to the cause of downly mildew disease (Burhanuddin, 2009). The alternative for controlling this disease is by using natural controlling agent which is environmentally friendly such as *Trichoderma* spp. fungi which can be used to control downy mildew disease by functioning as biofungicide and improving plant resistance to pathogen by inducing corn plant resistance.

The most common known inhibiting type and working mechanism of *Trichoderma* spp. are micro-parasitism, space and nutrition competition, the toxin production of *Trichodermin*, *Gliotoxin*, and *Gliovirin*to degrade pathogenic cells so that they cannot develop, the plant resistance induction, sprouting metabolism stimulation and additional mechanism related to plant resistance against the disease (Howell, 2003). The target-specific *Trichoderma* spp. controlling mechanism which is colonizing rhizosphere immediately andprotecting roots from pathogenic fungal attack, accelerating the plant growth and improving plant production result, become the superiorities as a natural controlling agent.*Trichoderma* spp. is easy to propagate massively and easy to store in long term (Purwantisari and Hastuti, 2009). Based on these elaborations, a research was required to test *Trichoderma* spp. fungi effectiveness as biofungicide and inducer of corn (*Zea mays* L.) plant against downy mildew disease.

The objective of this research was to find out the effectiveness of *Trichoderma* spp. as biofungicide and inducer of corn plant resistance.

2. Materials and Method

This research was conducted from May to July 2018, in the Plant Pest and Disease Laboratory of Agrotechnology Department of Faculty of Agriculture in Lampung University. This research used a completely randomized design (CRD) with 16 treatments and repeated 3 times with a total of 48 units of trials. The treatments were without treatment (0), *Trichoderma* spp. GDR isolate (1) *Trichoderma* spp. NTF isolate (2), and *Trichoderma* spp. TRJ isolate (3) which were applied to the plant growing points as fungicide (B) and as inducer of plant resistance to be applied in the plant roots (P).

Obtained data were tested for homogeneity by using Bartlett test and Tukey test to test additions. When the assumption was satisfied, data were analyzed by using analysis of variance and middle value comparison between treatments was tested by using Scheffe test in 5% trust level. In Scheffe test, treatments were grouped as they are shown in Table 1 and Table 2.

Table 1. Group/structure of *Trichoderma* spp. treatments as biofungicide and corn plant resistance inducer against downy mildew disease at Scheffe test

Group/ Structure ofTreatments	Description	Symbol
A	Control	B0P0
B	*Trichoderma* spp. isolate treatments at growth points	B1P0, B2P0, B3P0
C	*Trichoderma* spp. isolate treatments at roots	B0P1, B0P2, B0P3
D	*Trichoderma* spp. isolate similar treatments at growth points and roots	B1P1, B2P2, B3P3
E	*Trichoderma* spp. isolate different treatments at growth points and roots	B1P2, B1P3, B2P1, B2P3, B3P1, B3P2

Note: B0 = without biofungicide at growth points, B1 = GDR isolate biofungicide treatment at growth points, B2 = NTF isolate biofungicide treatment at growth points, B3 = TRJ isolate biofungicide treatment at growth points, P0 = treatment without inducer at roots, P1 = GDR isolate inducer treatment at roots, P2 = NTF isolate inducer treatment at roots, P3 = TRJ isolate inducer treatment at roots

Table 2. The main contrast of *Trichoderma* spp. treatments as biofungicide and corn plant resistance inducer against downy mildew at Scheffe test.

Comparison	Description	Symbol
A vs B, C, D, E	Control versus all treatments of *Trichoderma* spp.	B0P0 **vs** B0P1, B0P2, B0P3, B1P0, B1P1, B1P2, B2P3, B2P0, B2P1, B2P2, B2P3, B3P0, B3P1, B3P2, B3P3.
B, C vs D, E	*Trichoderma* spp. treatments at one of plant parts (either growth points or roots only) versus both of them (growth points and roots)	B1P0, B2P0, B3P0, B0P1, B0P2, B0P3 **vs** B1P1, B2P2, B3P3, B1P2, B1P3, B2P1, B2P3, B3P1, B3P2
B vs C	*Trichoderma* spp. treatments at growth points versus roots.	B1P0, B2P0, B3P0 **vs** B0P1, B0P2, B0P3
D vs E	*Trichoderma* spp. treatments at both plant parts (growth points and roots); the same isolate versus different isolate.	B1P1, B2P2, B3P3 **vs** B1P2, B1P3, B2P1, B2P3, B3P1, B3P2

Note: B0 = without biofungicide at growth points, B1 = GDR isolate biofungicide treatment at growth points, B2 = NTF isolate biofungicide treatment at growth points, B3 = TRJ isolate biofungicide treatment at growth points, P0 = treatment without inducer at roots, P1 = GDR isolate inducer treatment at roots, P2 = NTF isolate inducer treatment at roots, P3 = TRJ isolate inducer treatment at roots

Incubation time (day). The incubation time is the required by the plant from inoculation until early plant sicknesssymptom appears. Observation was done every day until early downy mildew disease symptom appeared.

Disease occurrence (%). Disease occurrence is the numbers of units of sick plants compared to all observed units. Unit here could be parts of a plant, a whole individual, or a clump of plant. The disease occurrence can be estimated by using a formula (Ginting, 2013):

$$\text{TP} = \frac{n}{N}\ x\ 100\ \%$$

Description:

TP = Disease occurrence (%)
n = unit of sick plant
N = observed unit of plant

Disease severity (%). Disease severity is defined as area or volume of sick plant tissue compared to all area or volume. Disease score and scaleare used to measure the plant disease severity.A disease is given a score according to the occurring severity level. The severer a disease then the higher will be the score and vice versa. Disease score or scale and formula of disease severity was modified based on Ginting (2013) reference, which is expressed as follows:

$$PP = \sum \frac{(n\ x\ v)}{N\ x\ V}\ x\ 100\ \%$$

Description:

PP : Disease severity (%)
n : numbers of leaf with certain score
v : Numeric value for each category of disease attack
N : Observed numbers of leaf(sample)
V : Highest score or scale

Stover dry weight (g).The plant was cut at the part between stem and root. The stover was then cut and dried with sunrays for 4 days. The dried stover was entered into envelops to be entered into oven with 80^{o}C temperature for 7 days until the stover weight was constant.

3. Result and Discussion

3.1 Symptoms of corn downy mildew disease

The downy mildew disease symptom was started with leaf with chlorosis or whitey color extending parallel to the leaf bone (Figure 3A). This symptom appeared most early at 4 days after inoculation of *Peronosclerospora* sp. fungi. There was conidium layer of fungi with white color like powder on leaf surface (Figure 3B).

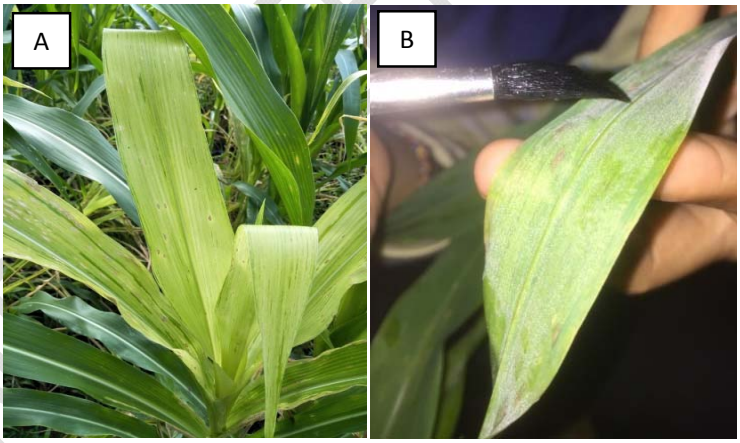

Figure 3. The symptom of downy mildew disease: (A) leaf chlorosis, (B) conidium layer fungi with white color like powder on leaf surface.

3.2 Incubation time of downy mildew disease

The *Trichoderma* spp. fungi treatment result as biofungicide and plant resistance inducer affected in extending the downy mildew incubation time (Table 4).

Table 4. The downy mildew disease incubation time (day) with*Trichoderma* spp. treatment as biofungicide and plant resistance inducer

No	Treatment Group	Description	Incubation time	F-hit Scheffe	F-table
1	A vs B, C, D, E	Control versus all*Trichoderma* spp. treatments	4,0 vs 4,6	6,84 *	2,01
2	B, C vs D, E	*Trichoderma* spp. treatment at either part of plant (growth point or root only) versus *Trichoderma* spp. treatment at both parts of plant (growth point and root)	4,2 vs 4,9	30,14 *	2,01
3	B vs C	*Trichoderma* spp. treatment at growth point versus *Trichoderma* spp. treatment at root	4,1 vs 4,3	1,62 tn	2,01
4	D vs E	*Trichoderma* spp. treatment at both growth points and roots with same isolate versus *Trichoderma* spp. treatment at both growth points and roots with different isolate	4,9 vs 4,9	0,10 tn	2,01

Note: * = significant, tn = not significant.

3.2 Occurrence of downy mildew disease

The occurrence of downy mildew disease is the percentage of sick plant compared to all observed plants in a certain area. The *Trichoderma* spp. fungi treatment as biofungicide and plant resistance inducer affected in reducing the downy mildew disease occurrence at 4 and 5 days after inoculation (DAI) (Table 5).

Table 5. Downy mildew disease occurrence (%) with *Trichoderma* spp. treatment as biofungicide and resistance inducer of corn plant

No	Treatment Group	Description	Disease occurrence (%)		F-hit Scheffe		F-tabel
			4 DAI	5 DAI	4 DAI	5 DAI	
1.	A vs B, C, D, E	Control versus all *Trichoderma* spp. treatments	33,27 (1,72) vs 12,35 (1,44)	61,87 (7,85) vs 45,98 (6,65)	7,40 *	2,59 *	2,01
2.	B, C vs D, E	*Trichoderma* spp. treatment at either part of plant (growth point or root only) versus *Trichoderma* spp. treatment at both parts of plant (growth point and root)	26,14 (1,62) vs 3,16 (1,32)	62,66 (7,89) vs 34,86 (5,82)	33,60 *	29,90 *	2,01
3.	B vs C	*Trichoderma* spp. treatment at growth point versus *Trichoderma* spp. treatment at root	26,92 (1,66) vs 29,72 (1,63)	65,04 (8,03) vs 60,28 (7,76)	0,99 tn	0,21 tn	2,01
4.	D vs E	*Trichoderma* spp. treatment at both growth points and roots with same isolate versus *Trichoderma* spp. treatment at both growth points and roots with different isolate	1,58 (1,30) vs 3,96 (1,33)	38,03 (6,08) vs 33,27 (5,68)	0,17 tn	0,61 tn	2,01

Note : DAI = day after inoculation; * = significant, tn = not significant, number in parenthesis is the transformation result of $\sqrt{(x + 0,5)}$.

3.4 Downy mildew disease severity level

The result of analysis of variance of all *Trichoderma* spp. fungi treatments as biofungicide and plant resistance inducer showed that they were not able to suppress the downy mildew disease severity level at 7, 14, and 21 days after inoculation (DAI) (Table 6). The middle value separation test with Scheffe test at 5% was not conducted because F_{count} value at the analysis of variance was lower than F_{table} value (not significant).

Table 6. The analysis of variance of downy mildew disease severity level with *Trichoderma* spp. treatment as biofungicide and corn plant resistance inducer

Analysis of Variance	F-count			F-table
	7 DAI	14 DAI	21 DAI	
Treatment	0,73 tn	0,54 tn	0,84 tn	2,01

Note : DAI = Day after inoculation; * = significant, tn = not significant.

Stover dry weight

The result of analysis of variance of all *Trichoderma* spp. fungi treatments as biofungicide and plant resistance inducer showed that they were not able to improve the stover dry weight of corn plant with downy mildew disease (Table 7).The middle value separation test with Scheffe test at 5% was not conducted because F_{count} value at the analysis of variance was lower than F_{table} value (not significant).

Table 7. The analysis of variance of corn plant stover treated with *Trichoderma* spp. as biofungicide and corn plant resistance inducer against downy mildew disease

Analysis of Variance	F-count	F-table
Treatment	1,21 ^{tn}	2,01

Note : * = significant, tn = not significant.

The research results showed that the early downy mildew disease symptom appeared at 4 days after inoculation or 14 days after planting. The symptom was leaf with chlorosis or whitey color extending in parallel along the leaf bone (Figure 3A). There was fungal spore layer with white color like powder on the leaf surface (Figure 3B), and these fungi were very visible in the morning. The symptom was systemic because it attacked growth points and spread to all plant parts. This is in line with Semangun (2004) who suggests that the downy mildew disease is able to cause spreading systemic symptom extending to all plant parts or able to cause local symptom. The systemic symptom only occurs when fungus in the infected leaf can reach growing points so that they will infect all leaf formed by the growth points. At 2-3 weeks plant, the stem growth is inhibited, the color goes into yellowish, and there are fungal conidium layers with white color at underside of the leaf.

The *Trichoderma* spp. as biofungicide and plant resistance inducer was able to extend incubation time of downy mildew disease (Table 4) and reduced disease occurrence at 4 and 5 days after inoculation (Table 5). The *Trichoderma* spp. treatment in this research could only reduce the disease occurrence at 4 and 5 days after inoculation. The research result of Sutama *et.al.* (2015) suggests that the *Trichoderma* spp. application could reduce the disease occurrence of downy mildew disease at hybrid corn NK22 only at 33 and 40 days after planting. This might be because the severe pathogen attack level from natural pathogen inoculation caused by climate factor such as air humidity and temperature. This factor was strengthened by the continuous rainy climate condition when inoculation was conducted.

Trichoderma spp. is able to serve as a biofungicide in extending the incubation time and to reduce disease occurrence of downy mildew by inhibiting the *Peronosclerospora* sp. fungus

growth that cause downy mildew disease by competing aggressively or occupying unoccupied spaces. This is in line with Herlina (2009) who suggests that the *Trichoderma* spp. as an active biofungicide fungal agent has micro-parasite nature, which inhibits other fungal growths by parasitism mechanism. The occurring mechanism is that the *Trichoderma* spp. fungal growth runs twice faster so that they shall coil surrounding pathogen fungal hypha. Along with coiling and surrounding these hyphae, enzyme capable of modifying cellular walls of pathogenic fungal hypha is released.

Trichoderma spp. serving as inducer of plant resistance is also proven to be able to induce actively plant resistance gens from being passive into active. This is in line with Oanh *et.al.* (2006) who suggests that *Trichoderma* spp. fungus can improve plant resistance by activating resistance gens in the plant. The *Trichoderma* spp. fungus applied to corn plant roots can trigger peroxide enzyme activity serving to strengthen cellular walls against enzyme degradation produced by pathogen through formation of structural protein in cellular walls. Peroxide is an enzyme serves as catalysator at final process of lignin biosynthesis and hydrogen peroxide processes.

This result is strengthened with Davis (2010) who states that induced resistance commonly is systemic, because resistance ability is improved not only in the infected plant parts, but also in uninfected plant parts. Some *Trichoderma* spp. strains have potentials as influencers that produce systemic resistance reactions in the plant. One of produced resistance reactions is improvement of chitinase enzyme in the plant tissues. Therefore, the *Trichoderma*spp. fungal isolate can reduce the disease occurrence of downy mildew disease at corn plant through mechanism of plant resistance induction.

Induced systemic resistance can be made as an alternative to obtain genetic diversity, especially the plant resistance character against a disease. The systemic resistance induction is a process to stimulate resistance of host plant without introduction of new genes. Systemic resistance induction causes a physiological condition that regulates to activate resistance system and stimulate natural resistance mechanism owned by the host by applying external induction materials. External inducer materials can be biological, chemical, and physical agents (Agrios, 2005).

Plant resistance improvement by induction can be done by Systemic Acquired Resistance (SAR) process or Induced Systemic Resistance (ISR) that involve varying types of genes,

enzymes, and proteins. According Pieterse *et.al.* (2009), the plant resistance improvement by SAR occurs after a pathogen infection locally at the plant, and then the infected plant activates genes serving for resistance (pathogenic related genes) that produce chemical compounds for plant defense, salicylate acid and PR-protein group like peroxides. When the plant has been induced for its resistance, and then being infected again by other pathogens, the plant will be able to defend itself against undeveloped or localized pathogen infection because the plant cells around infected site die. The cell death in this process is commonly referred to as hypersensitive reaction. Meanwhile, the trigger of resistance by SAR occurs not because of pathogenic infection, but by *Trichoderma* spp. infection.The plant responses the *Trichoderma* spp. infection, and then the plant produces plant defense compounds such as jasmonic acid and ethylene compounds.

Trichoderma spp. can also stimulate the formation of varying compounds that are able to induce plant resistance both locally and systematically against pathogens (Harman *et.al.,* 2004). In inducing plant resistance, *Trichoderma* spp. had been reported to be able to activate jasmonic acid and ethylene compound signal paths (Saksirirat *et.al.,* 2009). The potential of some *Trichoderma* spp. strains in inducing plant resistance has been widely reported. *Trichoderma harzianum*T39 induces bean plant resistance (De Meyer *et.al.,* 1998) and other *Trichoderma* spp. are able to induce resistance for varying plants against different diseases.

Trichoderma spp. serving as biofungicide and plant resistance inducer ins not influential to reduce disease severity and to improve plant stover dry weight. It is suspected because unsuccessful efficacy of *Trichoderma* spp. formulation as soil infectious fungal controller is determined by some factors including soil humidity, types of soils, method and application (Dini, 2016 in Faishol *et.al.,* 2018). According to Chatri *et.al.* (2018), natural agent before being introduced into soil should be propagated massively in organic materials according to growth and development so that it will make adaptation with a new environment after being introduced into the soil. The antagonist fungal growth is very depending on input of energy and nutrition that are commonly available in the planting media. Antagonist fungus obtain energy and nutrition from organic material decomposition in the soil and use it for their activities and propagation of the population.

According to Ginting and Maryono (2012), to obtain effective antagonist fungus in controlling plant disease, the antagonist fungus must have good quality. The antagonist fungus quality is determined by numbers of formed propagule (conidia) and percentage of

fungal propagule growth. To obtain good quality fungus, a supporting media is required to improve numbers of propagule and its growth, one of them is rice grain organic material (Ginting and Maryono, 2011). A research done by Gusnawaty *et.al.* (2017) indicates that the *Trichoderma* spp. propagation in rice bran media is better. It is because more nutrition content in rice bran media and it is easier to degrade by *Trichoderma* spp. fungus, so that the numbers of *Trichoderma* spp. spores are much more compared to other propagation media. According to Gusnawaty *et.al.* (2014), the rice bran nutrition content is very suitable for *Trichoderma* spp. fungal sporulation and higher sporulation process will produce more numbers of spores, and vice versa.

4. Conclusion

The conclusion of this research is that the *Trichoderma* spp. treatment as biofungicide and plant resistance inducer is effective to extend incubation time and to reduce disease occurrence of downy mildew disease significantly at the early course of the disease.

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