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Trichoderma and Bacillus as Combined Biocontrol Agent of Moler Disease on Shallots

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ABSTRACT

Moler disease of shallot (MDS) or basal plate rot caused by *Fusarium oxysporum* f.sp. *capae* (Foce) is an important disease on shallot. In low land such Brebes Central Java where is one of the center production of shallots in Indonesia, the disease is the most important and harmful. The specific symptom of the disease is a twisting leaf so the farmers call it as "Moler" meaning twisting. In the fields, the disease intensity can reach over 70%. So far the disease is still difficult to control. The control development of MDS is important to do. It is understood well that biological control of disease is promising for the future of plant production. The effectiveness consistence however is one of problem in single application of biological control agent. The problem is caused by varies environmental condition in the fields that are not always favourable to biocontrol agents. To minimize the risk of failure to control, it is necessary to use the controlling agent in an integrated or combination manner. Trichoderma and Bacillus were two biocontrol agents having been studied to control plant diseases showing effective to reduce disease intensity of MDS. The research aimed to study the effectiveness of Trichoderma and Bacillus to control MDS in a combination manner. The works in vitro were conducted in laboratory of Plant Pests and Diseases, whereas the works in vivo were arranged by completely randomized design (CRD) with 360 plant samples. The data were analyzed by Duncan's test. The results showed that the application of Trichoderma and Bacillus in vitro had a significant effect on decreasing the growth of pathogenic Fusarium and both microorganisms were antagonistic each other. In vivo however, the antagonism was not significant. The effectiveness of *Trichoderma* sp. and *Bacillus* sp. to control MDS singly in vivo reached up to 34.42% and 24.76% respectively. The implication is that the couple biological control agent is possible to apply in combination manner to minimize the risk of failure in single manner application.

Keywords: *Shallot, Trichoderma, Bacillus, Fusarium*

1. INTRODUCTION

Fusarium wilt of shallot (MDS) caused by pathogenic fungi of *Fusarium oxysporum* f. sp. *capae* (Foce). That is one of the main factors constrains the shallot plant growth and productivity. One of the most important and common diseases in shallot which caused a lot of losses in several production centers was MDS[1]. Damage caused by Foce can reduce yield up to 50%[2]. In low land, the pathogen causes a twisting symptom so the farmers call it as "Moler" meaning twisting. Foce included as soil-borne pathogen, Soil-born pathogen can survive in the soil for extend period of time where they remain dormant until they find a suitable host plant[3]. This pathogen caused a serious damage for large number.

To reduce the losses that caused by Foce, there are two alternative manner called chemical and biological control. Chemical disease control is not recommended because it strongly contaminate the environment and caused damage in natural balance if used continuously[4].

However Biological control agents (BCA) application is the alternative solution to used safely. Trichoderma sp. and Bacillus sp. are well known as the biocontrol agents for attacking Fusarium oxysporum. Trichoderma sp. is able to inhibit the growth of Fusarium sp. up to 49.7%[5]. Bacillus sp. is a bacterium that produces bacteriocyn and capable to kill target of pathogenic cells[6]. Single application of BCA is possible to used but highly risk with unsuitable environmental condition. In the other hand, single BCA may get failure in application manner, so the antagonist agents can not work effectively.

Another approach to improve the reliability and level performance of BCA is combining two biocontrol agents. Using mixture of antagonistic bacteria and fungi, and this approach has proven to be more effective than single strain treatments against a variety of plant disease[7]. Besides, it can be minimize the failure of single BCA application that caused by

unfavorable environmental condition. This research aimed to study the effectiveness of Trichoderma and Bacillus to control MDS in a combination manner.

2. MATERIAL AND METHODS

The research was conducted in laboratory of Plant Pests and Diseases Faculty of Acriculture, Universitas Sbelas Maret (UNS) in Surakarta, Indonesia. The antagonism tests were done by using dual culture method, whereas the works in vivo were arranged by completely randomized design (CRD) with 360 plant samples. The data were analyzed by Duncan's test. Tools and materials are Petri dishes, ose needles, bunsen, Laminar Air Flow (LAF), wrap, PDA, NA, *Trichoderma* sp., *Bacillus* sp. B16, *Fusarium oxysporum* f. sp. *capae* (Foce) and shallot. Trichoderma and Bacillus isolates were inoculated 7 days before planting, while Foce inoculated 7 days after planting. The variables consisted of inhibition rate, symptoms of disease, incidence of disease, severity of disease, rate of disease infection, and effectiveness of BCA in controlling disease.

3. RESULT AND DISCUSSION

Trichoderma and Bacillus were microorganisms that play role as BCA. The ability both of these antagonist agents is inhibiting the growth of pathogenic Foce. It was shown by the performance of each isolate at decreasing the number of Foce's colonies. Inhibition of BCA to Foce colonies is shown by Table 1.

Table 1. Growth decreases and colonies inhibition of Foce by *Trichoderma* and *Bacillus*

Biological Control Agents (BCA)	**Inhibition (%)* by**	
	Antagonism**	**VOCs*****
Trichoderma sp.	40.05a	63.33a
Bacillus sp. B16	47.39a	50.00b

*the number followed by same letter in the same column show the result are not significant by Duncan's test α: 0.05, ** dual culture test, ***Volatile Compounds produced by BCA.

*Trichoderm*a sp. and *Bacillus* sp. Inhibited significantly against Foce. Reliability of Trichoderma to inhibit the growth of Foce colonies reached to 40.05%. While Bacillus inhibited Foce colonies up to 47.35%. Antagonist hyphae that successfully intervene will absorb the essence of food, so that the pathogenic fungal hyphae can shrink and die[8]. Both of BCA released VOCs and effected to resistance of Foce growth up to 63.33% and 50%.

Trichoderma is able to grow better than the pathogens (column a.), this agents compete the growth area to inhibit Foce. There is not any separating zone between both of them. The hypae from Trichoderma intervene Foce, and it causes the agents can directly attack pathogen. Trichoderma sp. produce several antibiotics compounds such as alametichin, paracelsin, glioviridin, trichotoxin, which can destroy antagonist fungal cells through destruction of cell membrane permeability and inhibits many functional systems in making susceptible pathogens[9]. In the other hand, Bacillus has an antibiosis mechanism to against Foce (column b.), it can be seen by the formed of clear zone between both of them. This bacteria produce various types of protease enzymes and other enzymes that are able to degrade natural substrates and contribute to nutrient turnover[10], and also directly antagonize fungal pathogen by competing for niches and essential nutrients[11].

Inhibition formed on column c. and d. were carried out by volatility production from each BCA. It shows that Foce has more limited growth than antagonist agents. Trichoderma sp. having the ability to produce anti fungal effects indirectly on fungal growth[12], and the indication for Trichoderma to produce important metabolites (VOCs) that inhibit mycelium growth[13]. Bacillus sp. is the bacterium that produces anti fungal properties volatile organics compounds. These volatile arranged by some chemical compounds such as 12 benzene, alkyl, alcohol, ketones, aldehyde, and others that can inhibit to fungal growth functionally[14].

Trichoderma sp. and *Bacillus* sp. were incompatible each other. Allegedly, *Bacillus* sp. provided a lysis mechanism for the fungus Trichoderma sp. Llysis mechanism can be recognized by the fungus color changes[5]. This mechanism formed by secondary metabolite of Bacillus and similar with lysis enzyme compound that evaporated easily and other toxic substance[15]. *Bacillus* sp. has antiseptic properties and degradation of starch[16].

The symptoms of MDS which is shown by shallots was the exist of twisting and wilting around the base leaf. Some of shallots impacted by decoloring and chlorosis, so the chlorophyl were not enough for photosynthetic process. The appearance symptoms of MDS shown by this Table 2.

Table 2. The first day of symptoms appearance of MDS that inoculated by *Trichoderma* sp. and *Bacillus* sp.

Treatments	**symptoms Appearance (DAP)***	**Number of plants**
No BCA	14	3
Trichoderma sp. (A)	13	3
Bacillus sp. B16 (B)	14	3
Combined A + B	11	6
Combined A+B with no Foce	14	2

*DAP: Day After Planting.

The first day appearance of symptoms that indicated by application of combination manner showed faster at 11 DPA with 6 plants. Presence of many biological inputs to plant can caused biotic stress that disrupt plant growth. Sstress both biotic and abiotic can inhibit physiological growth in a plant[17]. Based on the Table 2. the rate appearance of early symptoms

occurred in the second week. Fusarium has spreading pathways through wind, irrigation, and agricultural equipment, symptoms of MDS existed around 2-3 weeks after planting[18].

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Table 3. Disease Incidence and severity decreases of MDS on shallot inoculated by *Trichoderma* sp. and *Bacillus* sp.

Biological control Agents (BCA)	**Disease Incidence (%)**	**Disease Severity (%)**
No BCA	90.00c	86.67c
Trichoderma sp. (A)	78.33b	70.00b
Bacillus sp. B 16 (B)	78.33b	59.33a
Combined A+B	78.33b	70.67b

Description: the number followed by same letter in the same column show the result are not significant by Duncan's test α: 0.05.

MDS spread quickly at all manner of BCA application. However, common environment conditions very impacting, while these conditions around the plants were rainy season with high humidity, less of sun intensity, and less of optimum air temperature. M macro climate in rainy season caused moisture increased and also impacted to Fusarium virulence behavior[19]. That factors affected to incidence of disease, severity of disease, and rate of infection. Each data can be recognized by Table 3. and 4.

Table 4. shows the infection rate of MDS was not significantly different. However the highest rate of infection rate was indicated by without any BCA manner. Application of Bacillus sp. Given result reached to 0,053 per unit per week. The cause of pathogens high virulence was the presence of inoculum number, the resistance of host plant, and environmental weather factors that support the infecting[22]. Infection rate of MDS that applied by single application Trichoderma sp. and combination were sequenced by 0.026 and 0.036 per unit per week.

Based on the severity disease, the effectiveness percentage of BCA to attack pathogens was visualized by figure 1.

Table 4. Infection rates of MDS on shallot inoculated by *Trichoderma* sp. and *Bacillus* sp.

Biological control agents (BCA)	**Infection rate of MDS (per unit per week)**
No BCA	0.043a
Trichoderma sp. (A)	0.026a
Bacillus sp. B 16 (B)	0.053a
Combined A+B	0.036a

Description: the number followed by same letter in the same column show the result are not significant by Duncan's test α: 0.05.

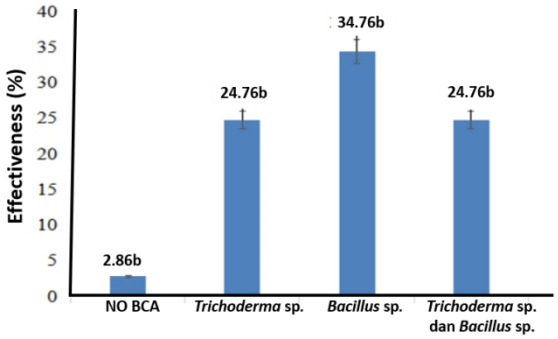

Figure 1. Control Effectiveness of severity disease by *Trichoderna* and *Bacillus* sp.

Effectiveness shown by single application of *Trihcoderma* sp. was not significantly different with combination manner, both of them had result number up to 24.76%. Single application of Bacillus sp. can control pathogens up to 34.42%. The higher effectiveness will impact to better BCA application in controlling disease[23].

4. CONCLUSION

Application of BCA had a significant effect in decreasing MDS. The highest control effectiveness to MDS reached by single application of Bacillus sp. up to 34.42%, while *Trichoderma* sp. and combination only 24.76%. However, application of combination manner between Trichoderma sp. and Bacillus sp. gave some number in reducing disease severity, so the implication of couple biological control agent is possible to apply in combination manner to minimize the risk of failure in single manner application.

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