Tropical Wetland Journal
Journal homepage: twj.ulm.ac.id | Published by Postgraduate Program - Lambung Mangkurat University | e-ISSN: 2654-279X

Original article
DOI 10.20527/twj.v7i2.92

Study of *Trichoderma spp*. Application Effects on Moler Disease Incidence and Its Effects on Shallot Growth and Yield

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Received: 28 December 2020; Accepted: 03 December 2021; Published: 17 December 2021

ABSTRACT

Fusarium wilt disease/moler is a concern in onion cultivation. Many farmers apply chemical pesticides such as azoxystrobin and difenoconazole to control the diseases. Both of these chemical pesticides are currently exhibiting a decline in effectiveness, prompting farmers to increase the dosage and frequency of application. Recommendations for biological control, including *Trichoderma spp..*
This study aimed to determine the influence of application time of of *Trichoderma spp.* in various media types on the incidence of moler disease and shallot growth and yield. This study was conducted in Tabalong district, South Kalimantan, from February to June 2019 using a completely randomized design (CRD) factorial comprising two nested designs. The first factor was the type of media in which *Trichoderma spp..* was applied, namely solid and liquid. The second factor was the application time of *Trichoderma spp..* Level 1 was the application of *Fusarium sp*. seven days before planting and *Trichoderma spp.*. seven days after planting. Level 2 included the application of *Trichoderma spp.*. seven days before planting and *Fusarium sp.* simultaneously during planting. Level 3 involved the administration of *Trichoderma spp..* concurrently with planting and *Fusarium sp.* seven days later. This investigation used one positive control (without treatment) and one negative control (shallots inoculated with *Fusarium*). Each experimental unit was consisted of ten plants and replicated three times. The parameters observed were the incubation period of *Fusarium sp*., disease incidence, number of leaves, plant height, number of cloves, and average clove weight. The study found that the application time of *Trichoderma* on various types of media affected the incidence of *Fusarium* disease, the number of leaves, and the number of shallot cloves.When compared to liquid media, the application of solid media resulted in a reduced incidence of *Fusarium* disease and a greater frequency of flares. The application time on solid media gave a low response to moles. As for liquid media, the lowest incidence of moler disease occurred at the seven days before planting.

Keywords: Biological agents, Shallot diseases, *Trichoderma* Application

1. Introduction

Shallots (*Allium cepa* var. ascalonicum L.*)* are one of the leading commodities that have been ntensively cultivated (Pusdatin Kemenkes, 2016). The demand for shallots is continuing to rise as the world's population grows. However, the production centers located in Central Java, East Java, West Java, and West Nusa Tenggara have been unable to keep up with the growing demand. As a response, the government has decided to expand the planting area outside of the production centers (Bappenas,

2015). Farmers are challenged with shallot diseases during the expansion of the planting area, one of which is Moler disease.

Fusarium sp. is a soil-borne pathogen that causes a Moler disease (Shofiyani & Suyadi, 2014). It is most frequently seen on shallot plants, where it causes root rot and twisting disease (commonly referred to as Moler) (Kalman et al., 2020), posing a considerable obstruction to shallot production (Isniah & Widodo, 2015).

Biological agents are recommended to control moler disease. *Trichoderma spp.* is known to have antagonistic ability against soil-borne pathogenic fungi, easily found in soil ecosystem and has been widely studied to control Moler disease. *Trichoderma spp.* enzymes could degrade the cell walls of the pathogenic fungus (Saba et al., 2012). (El-Mougy & Abdel-Kader, 2019)in vitro research found that *Trichoderma viride* had the highest inhibition against *F. oxysporum cepae*, up to 95%, whereas in the field, *P. fluorescens* had the highest (69.6%). Media type and application time of *Trichoderma spp*. have not been widely studied.

(Deden & Umiyati, 2017)only discovered that the optimal application dose of *Trichoderma spp.* for reducing *Fusarium* disease was 5 ml per liter liquid media for each planting hole. According to (Kannangara & Dharmarathna, 2017), using the antagonistic fungus *Trichoderma spp*. a week before *Fusarium sp*. may lower the prevalence of corn stem rot disease. Therefore, additional research on the media type and application time of *Trichoderma spp.* for the control of Moler disease is required.

This study aimed to analyze the effect of application time of *Trichoderma spp.* in different types of *Trichoderma spp.* media on the incidence of Moler disease and shallot growth and yield.

2. Materials and Methods

The research was conducted at the shallot planting location in Jaro village, Jaro sub-district, Tabalong district, South Kalimantan. The research took place over four months, from February to June 2019. The research activities included preparation, planting, harvesting, and results analysis. *Fusarium sp.* was isolated from the soil at the research site. *Trichoderma spp.* was isolated from the rhizosphere of bamboo plants at the same location and then replicated in the South Kalimantan BPTPH Laboratory. The soil was sterilized with water steam (Pinaria & Assa, 2017). Observation of the incubation period of *Fusarium sp.* was calculated from the shallot inoculation with *Fusarium sp.* until the symptoms developed. The observation incidence was calculated by the formula: I = (a/b) x 100%. "I" denoted disease incidence, "a" denoted diseased plants, and "b" denoted total plants. The counting of leaves and measuring plant height began seven days after planting and was repeated every seven days. The number of cloves and the average clove weight were determined during harvesting. Wet weight was calculated during harvest, and storage dry weight was calculated seven days later.

The research employed a completely randomized design (CRD) consisting of two nested factorial designs. The first factor was the media types of *Trichoderma spp.*, consisting of two levels, namely j1 (*Trichoderma spp.* in solid rice media, the dose of 40 kg ha^{-1}, the density of 106 spores ml^{-1}) (Ramadhina, Ramadhina, Lisnawita, & Lubis, 2013), and j2 (*Trichoderma spp.* in liquid potato and sugar extract, the dose of 45 kg ha^{-1}, the density of 106 ml^{-1} spores) (Baihaqi, et al., 2013). Factor II consisted of 3 levels: (1) *Trichoderma spp.* applied seven days after planting and *Fusarium sp.* seven days before planting (w1). (2) *Trichoderma spp.* applied seven days before planting and *Fusarium sp.* simultaneously at planting (w2). (3) *Trichoderma spp.* applied simultaneously at planting and *Fusarium sp.* seven days after planting (w3). This study examined six combinations, namely w1 (j1), w2 (j1), w3 (j1), w1 (j2), w2 (j2), and w3 (j1) (j2). Additionally, one control without treatment (-) and one control of shallot treated with *Fusarium sp*. (+) were included. The whole experiment was performed three times, resulting in 24 experimental units, each of which included ten plants. Parameters observed were the incubation period of *Fusarium sp.*, the incidence of disease, number of leaves, plant height, number of cloves, and average clove weight.

3. Results and Discussion

The application of *Trichoderma* to solid media for seven days after planting and *Fusarium sp.* seven days before planting (w1) experienced the emergence of *Fusarium* attack symptoms faster than the application in liquid media. While the application of *Trichoderma spp.* seven days before planting and

Fusarium sp. simultaneously at planting (w2) was more susceptible to *Fusarium* disease than in the solid media.

The application of *Trichoderma* did not affect the incubation period of *Fusarium* on shallot plants, both in solid (j1) and liquid (j2) media. However, if *Trichoderma* in liquid media was applied before planting (w2), the incubation period was shorter and significantly different from the seven days after planting (w1) and during planting time (w3) (Table 1).

Table 1. The variance analysis effect of media type and application time of *Trichoderma spp.*. on the incidence of Moler disease in shallots against all observed variables.

Variable	Trip			
	Media type (j)	Application times (w)	Positive vs Trip Control (K+ x P)	Negative vs Trip Control (K- x P)
MIF	ns	*	ns	Ns
IP	*	*	**	**
JD 1 MST	ns	**	**	**
JD 2 MST	ns	ns	**	**
JD 3 MST	ns	**	**	**
JD 4 MST	ns	ns	**	**
JD 5 MST	ns	ns	**	**
JD 6 MST	ns	ns	**	**
JD 7 MST	ns	ns	**	**
JD 8 MST	ns	ns	**	**
JD 9 MST	ns	ns	**	**
JS	*	ns	ns	Ns
TT 1 MST	ns	ns	ns	Ns
TT 2 MST	ns	ns	ns	Ns
TT 3 MST	ns	ns	ns	Ns
TT 4 MST	ns	ns	ns	Ns
TT 5 MST	ns	ns	ns	Ns
TT 6 MST	ns	ns	ns	Ns
TT 7 MST	ns	ns	ns	Ns
TT 8 MST	ns	ns	ns	Ns
TT 9 MST	Ns	ns	ns	Ns
BB	Ns	ns	*	*
BK	Ns	ns	*	*

Information: MIF = Incubation Period; IP = Incidence of Sisease; JD = Number of Leaves; JS = Number of Clove; TT = Plant Height; BB = Wet Weight; BK = Dry Weight; WAP = Weeks After Planting; P = Treatment; j = Type of *Trichoderma spp.* Application Media; w = *Trichoderma spp.* Application Time; K + x P = Positive control and treatment interactions; K- x P = Negative control interaction and treatment; *,= Has a significant effect according to the F test at the 5% significance level.; ** = According to the F test at 1% significance level, it has a very significant effect, and ns = no effect.

The incubation period is the time from inoculation to the onset of initial symptoms. According to (Fadhilah, et al., 2014), the earliest symptoms of *Fusarium* twisted disease in shallots during the growth period include observable indicators such as leaflets that begin to circle before turning yellow or changing color. Farmers on the Indonesian island of Java named it Moler, which means round, as a result of these symptoms.

The single factor, which was the types of media, did not significantly affect the incubation period. The application time factor (w) in liquid media had a significant effect on the incubation period. According to (Putra, Phaabiola, & Suniti, 2019), the delay in symptom emergence is caused by the continual conflict between pathogens and antagonistic agents, which results in pathogens infecting plants more slowly. Research by (Sunarwati & Yoza, 2010) stated that one of the crucial factors determining the activity of antagonistic microorganisms that can control pathogens is to have a high growth rate to compete with pathogens in terms of food and space control. As a result, they are capable

of inhibiting the growth of pathogenic fungus. When applied seven days before planting (w2), *Trichoderma* in liquid media had an opportunity to adapt to its environment prior to encountering the *Fusarium*. Thus, it is believed that the ability of *Trichoderma* and *Fusarium* to compete for space and nutrients in the shallot rhizosphere is developing faster than when *Trichoderma* directly meets the pathogens.

Based on the analysis of variance on the incidence of *Fusarium* disease (Table 1), it shows that the application time and media type of *Trichoderma spp.* (J) on the control had a very significant effect. The single factor of media type and application time had a significant effect. It proves that the application of *Trichoderma* in this study could reduce the incidence of Moler disease (Table 4). It has also been confirmed by (Ramadhina et al., 2013) and (Akhtar & Javaid, 2018). According to (Deden & Umiyati, 2017), *Trichoderma* could limit *Fusarium oxysporum f. sp. cepae* growth by up to 95.9 percent in vitro. In field conditions, *T. viride* could reduce disease up to 61.8%. Chemical fungicides only decreased disease by 22.22% under the same conditions. According to (Dendang, 2015), *Trichoderma* produces ß-(1-3)glucanase and chitinase enzymes. It induces pathogen exolysis and degrades the cell walls of *Fusarium*.

Solid media were more effective than liquid media at reducing disease incidence (Table 2). It is most likely due to rice's solid media, providing more steady nutrients than the liquid medium.

Table 2. The average difference test of the effect of *Trichoderma spp.* application time in media type on incubation period (days)

Times	Media types	
	j1	j2
w1	6,70 a	28,50 b
w2	4,27 a	7,93 a
w3	7,20 a	17,67 b
Average Type	18,17	54,10

Information: The mean with the same superscript sign in each column shows no significant difference based on the DMRT test at a significance level of 5%.

The mean difference test (Table 3 and Table 4) shows that the application time between *Trichoderma* in solid media and the incidence of shallot disease was not statistically different. However, the application time of *Trichoderma* in liquid media was significantly different at seven days after planting (w1) and at planting time (w3). *Trichoderma*'s ability to control *Fusarium* may occur because it is applied before getting the disease. It could cover a large portion of the root surface and win the competition for space and nutrients. As a result, pathogens become suppressed when infecting the plant roots. The high incidence of disease at seven days after planting and at the time of planting is thought to be caused by the availability of high initial inoculum

Table 3. The average difference test of the effect of *Trichoderma spp.* application time in media types on the incidence of disease attack (%)

Times	Media types	
	j1	j2
w1	36,67 a	83,3 b
w2	13,33 a	30,0 a
w3	23,3 a	83,3 b
Average Type	24,43	65,53

Information: The mean with the same superscript sign in each column showed no significant difference based on the DMRT test at a significant level of 5%.

Table 4. The mean difference test of the treatment effect compared to the control on the incidence of attacks.

Trip	incidence of attacks (%)

Control	100 b
Trip	61,67 a

Based on Table 5, the number of shallots inoculated with *Trichoderma* has a significant effect than the shallot inoculated with *Fusarium* (control-). Significant differences were seen at all ages, with an average difference of 5 to 13 leaves. Shallot applied with *Trichoderma* had more leaves than those without *Trichoderma* application. According to (Ramadhina et al., 2013), *Trichoderma* bioactivator increases the number of plant leaves. (Taufik, 2011) obtained similar results, stating that *Trichoderma spp.* on pepper plants could increase the number of leaves. (Latifah & Soesanto, 2011) stated that *T. koningii* stimulated an increase in the number of leaves by producing various growth hormones.

Table 5. The average difference test data from the effect of *Trichoderma* application time in the type of media and control on the number of leaves

Trip	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Control	1,67a	4,07a	7,33a	10,67a	13,53a	16,10a	17,87a	18,27a	18,33a
Trip	5,87b	11,13b	15,29b	19,23b	22,99b	26,58b	29,22b	31,24b	32,01b

Information : WAP = Weeks After Planting

The mean difference test (Table 6) shows that the number of shallots applied with *Trichoderma* had a significant effect than those without the *Trichoderma* (control). According to (Saba et al., 2012), *Trichoderma* sp. could stimulate the breakdown of crop wastes from land agriculture, promoting fruit and seeds development. (Sutedjo, 2002)stated that overhauling plant residues could increase nutrient content, improve soil structure, and increase photosynthesis and plant growth rate. (Marsono, 2002) explained that nutrients affect plant growth.

Table 6. The average difference test data from the effect of *Trichoderma* application time in the type of media and control on the number of cloves

Trip	Number of cloves
Control	15,40a
Trip	13,00b

Based on the results of the mean difference test given in Table 7, it is known that the application of *Trichoderma* has no significant effect on the plant height. The height of shallot plants at the age of nine weeks after planting tended to be constant, probably due to the nearing harvest time. The shallot plants applied with *Trichoderma* had a constant height even though they were infected with Moler disease than those without *Trichoderma*. According to (Antara, 2015), *Trichoderma* influences plants by increasing their growth rate and nutrient uptake efficiency by releasing regulating hormones.

Table 7. The average difference test data from the effect of *Trichoderma* application time in the type of media and control on plant height

Trip	1 WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP	7 WAP	8 WAP	9 WAP
Control	9,27	17,91	22,85	28,11	33,17	37,65	40,68	40,97	41,33
Trip	9,67	17,20	21,66	26,00	30,33	33,90	36,86	39,30	32,01

Information : WAP = Weeks After Planting

The mean difference test (Table 8) reveals that *Trichoderma* treatment had a significant influence on shallot yields compared to shallot plants that were not treated (control -). (Sihombing, Setiado, & Hasyim, 2013) stated that *Trichoderma* could increase production yields by adding organic matter in the soil. Additionally, (Ismail & Tenrirawe, 2011)demonstrated that the combination of *Trichoderma sp*. and compost could inhibit the development of *Fusarium* wilt, hence increasing

productivity. (Sumarni, Sopha, & Gaswanto, 2010) reported that incorporating *Trichoderma* into compost production enhanced phosphate and potassium levels, benefiting bulb filling.

Table 8. The average difference test data from the effect of *Trichoderma* application time in the type of media and control on the weight of shallot bulbs

Trip	Gross weight (g)	dry weight (g)
Control	266,24 b	229,45 b
Trip	168,19 a	144,96 a

4. Conclusions

The application time and media of *Trichoderma spp.* were significantly affected the incubation period, incidence of moler disease, the number of leaves of one WAP and three WAP. Howeever, it was not significantly different in the number of leaves of two WAP, the number of leaves of four to nine WAP, the number of shoots, plant height, tuber wet weight, and tuber dry weight. Solid media in the *Trichoderma* application significantly affected the incidence of moler disease and number of shoots, but not significantly different on incubation period, number of leaves, number of cloves, plant height, tuber wet weight, and tuber dry weight. The best application time for *Trichoderma spp.* on solid and liquid media was in the pre-planting period because it resulted in the lowest incidence of mole disease. Meanwhile, at the time of planting and after planting, the best type was solid media.

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