

EFFECTIVENESS OF THE APPLICATION OF ORGANIC MATTER AND *TRICHODERMA VIRIDE* FROM SUPPRESIVE SOIL TO CONTROL FUSARIUM WILT ON BANANA PLANT

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ABSTRACT

Effectiveness of the application of organic matter and *Trichoderma viride* from suppressive soil to control fusarium wilt on banana plant. Fusarium wilt disease caused by *Fusarium oxysporum* f.sp. *cubense* is one of the problems in banana production. This research was aimed to evaluate the effect of *Trichoderma viride* isolated from suppressive soil and organic matter on controlling fusarium wilt on banana plant. The efficacy trial consisted of 12 treatments and four replications within each treatment. Each experimental unit consisted of 10 plants. Organic matters used were rice straw, cassava peel, and compost. Treatments were arranged in a randomized complete block design. Data obtained were analyzed by analysis of variance followed by orthogonal analysis (Pd^{*}0.05). Application of *T. viride* suppressed disease incident up to 65%. The highest disease incident occurred in plants treated without *T. viride* and organic matter and in plants treated with organic matter only (78.33%). Disease incidence in plants treated with *T. viride* was lower than those treated with the combination of *T. viride* and organic matter. Different application times (in nursery or soil medium) did not significantly affect fusarium wilt incidence. The type of organic matter did not significantly affect the incidence and severity of fusarium wilt.

Key words: biological control, *Fusarium oxysporum* f.sp. *cubense* (Foc), fusarium wilt, organic matter, *Trichoderma viride*

ABSTRAK

Efektivitas aplikasi bahan organik dan *Trichoderma viride* dari tanah supresif untuk pengendalian penyakit layu fusarium pada tanaman pisang. Penyakit layu fusarium yang disebabkan oleh jamur *Fusarium oxysporum* f.sp. *cubense* (Foc) merupakan salah satu masalah dalam budidaya pisang. Penelitian ini bertujuan untuk mengevaluasi pengaruh kombinasi jamur *Trichoderma viride* yang berasal dari tanah supresif dan bahan organik terhadap penyakit layu fusarium pada pisang. Uji efikasi *Trichoderma* spp. terhadap penekanan perkembangan penyakit layu fusarium terdiri atas 12 perlakuan dan empat ulangan, setiap ulangan terdiri atas 10 tanaman. Perlakuan disusun dalam rancangan acak kelompok lengkap. Bahan organik yang dipakai ialah masing-masing jerami padi, kulit singkong, dan kompos. Data yang diperoleh diolah secara statistik dengan uji ragam dan dilanjutkan dengan analisis ortogonal kontras (Pd^{*}0,05). Aplikasi *T. viride* menekan keterjadian penyakit sebesar 65%. Keterjadian penyakit layu fusarium tertinggi terdapat pada perlakuan FT₀O₀ (tanpa pengendalian) sebesar 100% dan aplikasi dengan bahan organik saja sebesar 78,33%. Aplikasi *T. viride* saja memiliki keterjadian yang lebih rendah dibandingkan dengan kombinasi aplikasi *T. viride* bersamaan dengan bahan organik. Pengaruh aplikasi *T. viride* di pembibitan dan di media tanah tidak berbeda nyata terhadap keterjadian layu fusarium. Jenis bahan organik tidak berpengaruh nyata terhadap keterjadian dan keparahan penyakit.

Kata kunci: bahan organik, *Fusarium oxysporum* f.sp. *cubense* (Foc), jerami padi, kompos, kulit singkong, layu fusarium, pengendalian hayati, *Trichoderma viride*

INTRODUCTION

Fusarium wilt disease is known to be the most common problem in banana plantation in the world. The first internal symptom is a reddish-brown discoloration of the xylem and the external symptom is that leaves become yellow and brown then the plants eventually die. It is caused by *Fusarium oxysporum* f.sp. *cubense*

(Foc). This fungus is a soil borne pathogen and can be spread through soil. Its chlamidospores are able to survive for more than 30 years in the soil even though there are no host plant (Ploetz & Pegg, 2015).

Foc consists of three pathogenic races (Foc race 1, 2, and 4) and is classified according to their selective impairment of banana cultivars. Foc race 4 is economically important as it comprises strains that infect

Cavendish bananas, which are the most widely planted variety of bananas in the world, in both tropics (Foc TR4) and subtropics (Foc STR4) (Sutherland *et al.*, 2012).

Controlling the disease by using synthetic chemical fungicides could lead to resistance in fungal pathogens and result in fungicide residues that pollute the environment. In addition, the controlling action of the chemical is often late because it is generally based on symptoms such as wilt, whereas infection by soil borne fungi is often difficult to detect because the initial infection occurs at or below ground level. Therefore, the management of fusarium wilt should be integrated including the use of antagonistic organisms (biological agents) and organic matter (Otero *et al.*, 2014).

One of the potential antagonistic fungi used as a biological control agent is *Trichoderma* spp. Some studies show that *Trichoderma* spp. suppress the growth of *Fusarium oxysporum* (Christopher *et al.*, 2010; John *et al.*, 2010), *Colletotrichum acutatum* (Freeman *et al.*, 2004), *Rigidoporus lignosus* (Hutahaean & Junita, 2009), *Rhizoctonia solani* (Howell *et al.*, 2000), *Colletotrichum graminicola* (Harman *et al.*, 2004A), and *Phytophthora capsici* (Ahmed *et al.*, 2000). *Trichoderma* spp. suppress the development of pathogens by means of antibiosis, mycoparasitism, competition, and lysis (Dennis & Webster, 1971; Harman *et al.*, 2004b).

The use of organic matter such as animal manure, green manure (the incorporation of crop residues into the soil), composts and peats has been proposed, both for conventional and biological systems of agriculture, to improve soil structure and fertility (Magid *et al.*, 2001; Conklin *et al.*, 2002; Cavigelli & Thien, 2003), and decrease the incidence of disease caused by soil borne pathogens (Litterick *et al.*, 2004; Noble & Coventry, 2005).

Soil is a complex mix of organic and inorganic matter that includes thousands of different microorganisms, the vast majority of which are still undescribed. Some of the organisms are pests which cause significant crop losses while others perform 'environmental services' such as biological control of pests, aeration, drainage, nutrient and water cycling (Chandrashekara *et al.*, 2012). According to Cook & Baker (1983), disease suppressive soils are defined as soils in which the pathogen does not establish or persist, the pathogen establishes but causes no damage or the pathogen causes some disease damage but the disease becomes progressively less severe even though the pathogen persists in soil. The mechanism of suppression of soil includes antibiosis, competition, parasitism and

predation. This study was carried out with the aim of evaluating the effect of various organic matter types applied with or without combination with *Trichoderma viride* isolated from suppressive soil on controlling fusarium wilt (*Fusarium oxysporum* f.sp. cubense) on banana plant.

MATERIALS AND METHODS

Study Site. This research was conducted in Nusantara Tropical Farm Company (PT NTF) farm in Way Jepara, East Lampung and the Laboratory of Plant Pathology, Faculty of Agriculture, University of Lampung from January to June 2013.

Isolation and Pathogenicity Test of *Fusarium oxysporum* fsp *cubense* (Foc). Foc was isolated from root exhibiting symptoms by the tissue transplanting Method (Soytong & Quimio, 1989). Foc was incubated on potato dextrose agar (PDA) to get pure cultures. Isolates were identified by observation of morphological characteristics under a compound microscope. Isolates were tested for pathogenicity following Koch's Postulates (Grimes, 2006).

Preparation of *Trichoderma viride*. The *T. viride* isolates used were the most effective isolates in suppressing Foc growth according to the results of previous trial screening (Ivayani & Ginting, 2015). *Trichoderma* was isolated from banana plants rhizosphere. Soil was taken from healthy plant rhizosphere around the banana plants showing Fusarium wilt symptoms (suppressive soil). *T. viride* starter (formulation) was cultured in rice groats. Parboiled rice groats were put in a heat-resistant plastic then sterilized in autoclave for 1 hour at a pressure of 1.5 atm with a temperature of 121°C. Five mycelial plug (Ø0.5 cm) of the 4-day *T. viride* were inserted into each medium, then the entire medium was incubated at room temperature for 14 days accompanied by homogenizing after visible fungal growth.

Foc Inoculation. Foc was propagated in the rice groats medium (same method with preparation of *T. viride*) as inoculum for inoculating foc on two-month banana seedlings. Inoculation was done by making four holes around the plants as deep as 5 cm, with a distance of 2 cm from the base of the plant. After that, Foc preparation was placed into the hole as 2.5 g / hole or 10 g / plant.

Application of *T. viride* and Organic Matter.

Organic matters used were rice straw, cassava peel, and compost. Rice straw and cassava peel were cut into pieces or chopped and moistened. For *T. viride* application on two-week seedlings, 10 g of rice containing 1.73×10^8 *T. viride* /g spores were mixed with the media in the nursery by stirring evenly around the roots of the banana plant. After the banana seedlings were transferred to the soil medium, 100 g of organic matter were added. Planting media in the nursery in the form of husk, sawdust, manure, and coco-peat (coconut waste). For *T. viride* application on soil media, the treatment was performed simultaneously with the application of organic matter at the time the seedlings were two months old and transferred to the soil medium mixed with 10 g chopped rice containing 1.69×10^8 *T. viride* /g spores and 100 g organic matter.

Preparation of Plants and Planting Media. Banana plants were grown in 10-kg polybags with 10 kg soil as planting media 1 and were fertilized in accordance with the cultivation techniques in PT NTF (Nusantara Tropical Farm Company). Banana seedlings used were Cavendish seedlings which were susceptible to the Foc clone CJ30 (NTF company's clone).

Observation of Disease Incidence. Observation was done by observing the first symptom of yellowing leaves starting from the edges of old leaves after Foc inoculation until the 16 weeks after planting. After that, plant was uprooted to observe symptom on stumps (reddish brown discoloration).

Disease incidence in plants was calculated using the following formula:

$$\text{Disease incidence (DI)} = \frac{\text{number of infected plants}}{\text{total number of plant observed}} \times 100\%$$

Observation of Propagules Density. Observations on the density of *T. viride* and Foc propagules were performed at 6 and 12 weeks after inoculation (wai). Soil sampling was done on two replications, and from each treatment was taken two soil samples (two experimental units). Calculation of propagules density was done by multilevel dilution method. The soil around the rhizosphere of the plant was taken as much as 100 g at four points which was 10 cm from the banana plant stumps each of 25 g of soil, then the soil was put into plastic and stirred. In the laboratory, each soil sample was taken as much as 10 g of soil and put into an erlenmeyer flask and stirred with 90 ml of sterile distilled water for 30 minutes. Using a micropipette, 1 ml of the

soil suspension was inserted into another flask containing 99 ml of sterile distilled water to obtain 10^{-3} dilution. Similarly, a suspension was made up to a 10^{-5} dilution. A total of 0.5 ml suspension of diluted result was dispersed in petri dish containing potato dextrose agar supplemented with rosebengal, streptomycin and chlorophenicol (PDA-RSC) media then incubated for 3-5 days. The emerging colonies were calculated using a hand counter.

Experimental design and Data Analysis. Data obtained were analyzed using analysis of variance followed by orthogonal comparison analysis (Pd"0.05).

RESULTS AND DISCUSSION

Disease Incidence. Generally, the results of 13-16 weeks after inoculation (wai) showed that the treatment had significant effect on the incidence of fusarium wilt disease in banana plants (Figure 1). At 13-16 wai, no fusarium wilt disease (0%) was present in the FT₀O₀ treatment, i.e. treatment without Foc inoculation and without *T. viride* application nor organic matters. Furthermore, the treatment of FT₁O₀ (*T. viride* application only) also had a low disease incidence (25%). Compared with the FT₀O₀ treatment, *T. viride* in FT₁O₀ was able to reduce the incidence of fusarium wilt disease by 40% at 13, 50% at 14, 55% at 15 and 60% at 16. High disease incidence was also found in the treatment of FT₀O₁, FT₀O₂, and FT₀O₃ (organic matters alone) reaching 80, 67,5, and 70%, respectively.

Based on orthogonal comparison analysis (Table 1), it was seen that *T. viride* treatment was more suppressing compared to treatment without combination of *T. viride* application and organic matters and organic material application only. In addition, the combined application of *T. viride* along with organic matter showed higher disease incidence than *T. viride* application only. The effect of *T. viride* application time was not significantly different between applications in seedling and in soil media. In addition, the type of organic matter has no significant effect on disease incidence.

Observation on disease incidence was not only performed on leaves but also on stumps. Observation on the incidence of fusarium wilt disease based on symptoms on stumps was performed at 16 wai. The results of the observation showed that the average of the incidence in stumps was higher than in the leaves. This is presumably because the symptoms on the leaves are the result of symptomatic stumps. So even though the stump has been infected, during certain periods, the

symptoms on the leaves could not be seen until the incubation period.

Observation on the incidence of fusarium wilt disease based on symptoms on stumps (reddish brown discoloration) was performed at 16 wai. In contrast to

leaf observation, orthogonal comparison analysis showed that disease incidence in positive control (inoculated with FOC-without *T. viride* and organic matter) was significantly different from those with the treatments (*T. viride* and organic matter). *T. viride* treatment was

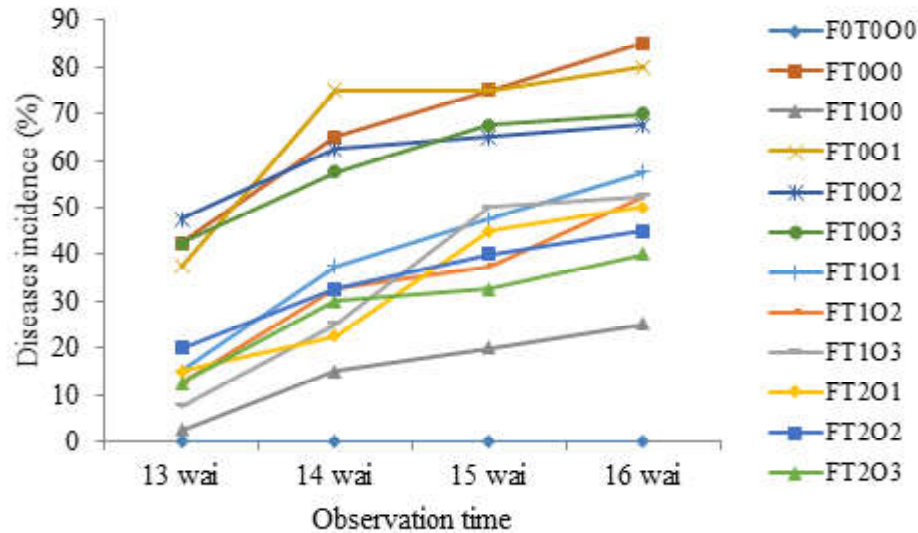


Figure 1. Disease incidence of Fusarium wilt based on leaves symptom
Remark: wai= weeks after inoculation; F₀= No Foc inoculation, F= Foc inoculation, T₀= no *T. viride* application, T₁= application of *T. viride* in seedling, T₂= application of *T. viride* in soil medium, O₀= no organic matter application, O₁= rice straw application, O₂= cassava peel application, O₃= compost application

Table 1. Orthogonal comparison of disease incidence of fusarium wilt based on leaves symptom

Comparison	F- value			
	Disease incidence			
	13 wai	14 wai	15 wai	16 wai
Replication	3.597 ^{ns}	6.373 *	7.082 *	5.785 *
Treatment				
1. T vs T + O	4.164 *	4.009 *	4.287 *	4.180 *
2. T vs O	23.973 *	19.533 *	14.256 *	10.914 *
3. O ₁ vs O ₂	0.998 ^{ns}	0.813 ^{ns}	0.393 ^{ns}	0.000 ^{ns}
4. O ₁ + O ₂ vs O ₃	0.000 ^{ns}	0.879 ^{ns}	0.32 ^{ns}	0.129 ^{ns}
5. O vs T + O	33.025 ^{ns}	25.524*	11.536 *	13.949 *
6. Ts + O vs Tm+ O	0.520 ^{ns}	0.173 ^{ns}	0.401 ^{ns}	0.129 ^{ns}
7. Ts + O ₁ + O ₂ vs Ts + O ₃	0.520 ^{ns}	0.694 ^{ns}	0.294 ^{ns}	0.653 ^{ns}
8. Ts + O ₁ vs Ts + O ₂	0.062 ^{ns}	0.130 ^{ns}	0.393 ^{ns}	0.217 ^{ns}
9. Ts + O ₁ + O ₂ vs Tm + BO ₃	0.332 ^{ns}	0.043 ^{ns}	0.524 ^{ns}	0.008 ^{ns}
10. Tm + O ₁ vs Tt + BO ₂	0.249 ^{ns}	0.529 ^{ns}	0.098 ^{ns}	0.605 ^{ns}
11. Non Treatment vs Treatment	8.201 *	6.402 *	5.211 *	5.925 *

Remark: T= *T. viride*, O= Organic matter, O₁= rice straw, O₂= cassava peel, O₃= compost, Ts= *T. viride* application in seedling, Tm= *T. viride* application in soil medium, wai= weeks after inoculation, *= significantly different (Pd^{0.05}), ns= non-significant

not significantly different from the combination of *T. viride* and organic treatments. The lowest incidence of fusarium wilt disease (0%) was found in the F₀T₀O₀ treatment, (without Foc inoculation and without the application of *T. viride* and organic matter). Furthermore, FT₁O₀ treatment (*T. viride* application only) also had low disease incidence up to 16 wai which is equal to 35%. Thus, *T. viride* treatment was able to

reduce the incidence of fusarium wilt disease by 65%. The best performance was found in FT₀O₀ (100%) treatment without *T. viride* application nor organic material. Furthermore, the highest disease occurrence was also found in the treatment of FT₀O₁, FT₀O₂, and FT₀O₃ (the application of organic matter only) which reached 85, 65 and 85%, respectively (Figures 2 and Table 3).

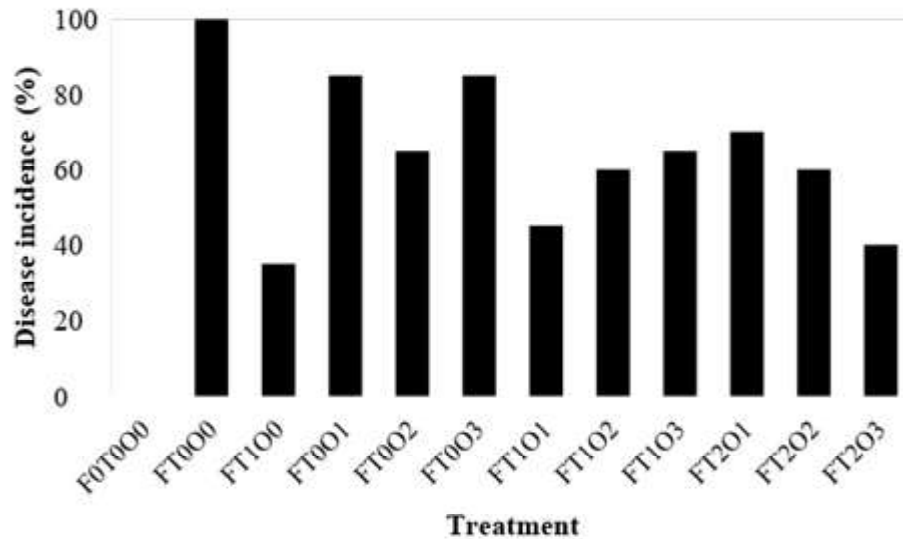


Figure 2. Disease incidence of Fusarium wilt based on stumps symptom

Remark: wai= weeks after inoculation, F₀= No Foc inoculation, F= Foc inoculation, T₀= no *T. viride* application, T₁= *T. viride* application in seedling, T₂= *T. viride* application in soil medium, O₀= no organic matter application, O₁= rice straw application, O₂= cassava peel application, O₃= compost application

Table 3. Orthogonal comparison of disease incidence of fusarium wilt based on stumps symptom

Comparison	F value
	Disease incidence
Replication	1.133 ^{ns}
Treatment	
1. T vs T + O	1.752 ^{ns}
2. T vs O	6.132 [*]
3. O ₁ vs O ₂	0.871 ^{ns}
4. O ₁ + O ₂ vs O ₃	0.291 ^{ns}
5. O vs T + O	4.208 [*]
6. Ts + O vs Tm + O	0.000 ^{ns}
7. Ts + O ₁ + O ₂ vs Ts + O ₃	0.453 ^{ns}
8. Ts + O ₁ vs Ts + O ₂	0.489 ^{ns}
9. Ts + O ₁ + O ₂ vs Tm + O ₃	1.814 ^{ns}
10. Tm + O ₁ vs Tt + O ₂	0.871 ^{ns}
11. Non Treatment vs Treatment	5.904 [*]

Remark: T= *T. viride*, O= Organic matter, O₁= rice straw, O₂= cassava peel, O₃= compost, Ts= *T. viride* application in seedling, Tm= *T. viride* application in soil medium;, wai= weeks after inoculation, *= significantly different (Pd^{0.05}), ns= non-significant

The use of organic matter such as animal manure, green manure (the incorporation of crop residues into the soil), composts, and peats has been proposed, both for conventional and biological systems of agriculture, to improve soil structure and fertility (Magid *et al.*, 2001; Conklin *et al.*, 2002; Cavigelli & Thien, 2003), and decrease the incidence of disease caused by soil borne pathogens (Litterick *et al.*, 2004; Noble & Coventry, 2005). Yet, data in this research showed that the application of organic matter alone was not able to decrease the disease incidence of fusarium wilt (Table 3). This was caused by the population of beneficial microbes in the organic matter was not enough to suppress the development of FOC. According to El-Sharouny (2015) & Bonilla *et al.* (2012), microbial populations of organic soil ameliorant were able to enhance plant health through disease suppression.

Propagules Density of *T. viride* and Foc. The highest propagules density of *T. viride* in the soil at 6 wai was observed in FT₁O₀ treatment (Table 4). Effects of *T. viride* application were significantly different from those of organic matter application (Table 5). However, at 12 wai, propagules density of *T. viride* in the soil in FT₁O₀ treatment was not significantly different from that in the combined treatments of *T. viride* and organic matter.

Different application times did not affect the propagule density of *T. viride*. These observational data were in agreement with the incidence of fusarium wilt disease. Propagules density of Foc was not significantly different between all the treatments, but the incidence of fusarium wilt disease was significantly different. This might happen because the plants applied with *T. viride* were more resistant than the plants with no *T. viride* application (Vitti *et al.*, 2016; Nawrocka & Malolepsza, 2013).

CONCLUSIONS

Application of *T. viride* suppressed disease incidence up to 65%. The highest disease incidence was observed in treatment of FT₀O₀ (100%) which was without *T. viride* and organic matter and in treatment of organic matter only (78.33%). The application of *T. viride* resulted in lower disease incidence compared to the combination of *T. viride* and organic matter. Different application time (nursery and soil medium) did not significantly affect fusarium wilt incidence. The type of organic matter did not significantly affect the incidence and severity of fusarium wilt.

Table 4. Propagule density of *T. viride* and Foc

Treatment	Propagule density (CFU/g)			
	6 wai		12 wai	
	T	F	T	F
	Original data			
F ₀ T ₀ O ₀	0	0	0	0
FT ₀ O ₀	3500	19000	5000	30500
FT ₁ O ₀	20500	10000	26000	20500
FT ₀ O ₁	3500	17000	7000	34500
FT ₀ O ₂	2500	17500	5500	36000
FT ₀ O ₃	2500	13000	4500	33500
FT ₁ O ₁	13500	13000	26000	22500
FT ₁ O ₂	13000	10500	28500	24500
FT ₁ O ₃	8500	9500	24500	22000
FT ₂ O ₁	10000	10500	24500	22000
FT ₂ O ₂	10500	13000	25000	21000
FT ₂ O ₃	7500	11500	25000	21500

Remark: T= *T. viride*, F= Foc, wai= weeks after inoculation, F₀= No Foc inoculation, F= Foc inoculation, T₀= no *T. viride* application, T₁= *T. viride* application in seedling, T₂= *T. viride* application in soil medium, O₀= no organic matter application, O₁= rice straw application, O₂= cassava peel application, O₃= compost application

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Table 5. ANOVA orthogonal contrast of propagule density of *T. viride* and Foc

Comparison	F value			
	6		12	
	T	F	T	F
1. T vs T + O	5.982 *	0.035 ^{ns}	0.003 ^{ns}	0.0274 ^{ns}
2. T vs O	24.945 *	0.496 ^{ns}	5.865 *	0.048 ^{ns}
3. O ₁ vs O ₂	0.174 ^{ns}	0.002 ^{ns}	0.045 ^{ns}	0.003 ^{ns}
4. O ₁ + O ₂ vs O ₃	0.058 ^{ns}	0.208 ^{ns}	0.097 ^{ns}	0.007 ^{ns}
5. O vs T + O	19.533 *	0.746 ^{ns}	14.982 *	1.34 ^{ns}
6. Ts + O vs Tm + O	0.738 ^{ns}	0.017 ^{ns}	0.023 ^{ns}	0.018 ^{ns}
7. Ts + O ₁ + O ₂ vs Ts + O ₃	1.398 ^{ns}	0.077 ^{ns}	0.042 ^{ns}	0.077 ^{ns}
8. Ts + O ₁ vs Ts + O ₂	0.011 ^{ns}	0.071 ^{ns}	0.024 ^{ns}	0.012 ^{ns}
9. Ts + O ₁ + O ₂ vs Tm + O ₃	0.579 ^{ns}	0.001 ^{ns}	0.001 ^{ns}	0.001 ^{ns}
10. Tm + O ₁ vs Tt + O ₂	0.013 ^{ns}	0.068 ^{ns}	0.001 ^{ns}	0.003 ^{ns}
11. No Treatment vs Treatment	4.163 *	0.653 ^{ns}	3.884 *	0.159 ^{ns}

Remark: T= *T. viride*, F= Foc, O= Organic matter, O₁= rice straw, O₂= cassava peel, O₃= compost; Ts= *T. viride* application in seedling, Tm= *T. viride* application in soil medium, wai= weeks after inoculation, *= significantly different (Pd>0.05), ns= non-significant

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ACCEPTED