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Growth Evaluation of Banana cv. Barangan as the Effect of *Trichoderma* sp. and Covering Types during Acclimatization Process

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Abstract

Acclimatization is one of the important processes in banana micro propagation before plantlets/ planting materials are ready to be cultivated in the field. *Trichoderma* sp. are well known as plant promoter fungi that can promote plant growth and increase survival rate of plantlets. The study was arranged in a Completely Randomized Factorial Design with two factors and four replications. The first factors was covering type i.e. 1) individual covering and 2) mass covering. The second factor was the proportion of *Trichoderma* addition into manure, namely a) manure without the addition of *Trichoderma* sp. (control), b) *Trichoderma* sp. : manure = 1 : 400 (w/w), c) *Trichoderma* sp. : manure = 1 : 800 (w/w) and d) *Trichoderma* sp. : manure = 1 : 1,200 (w/w). The purpose of this study was to investigate the effect of *Trichoderma* sp. applications and covering types in the growth of banana plantlets cv. Barangan during the acclimatization process. The results showed that the best treatment to induce plantlet growth during the acclimatization process was the addition of *Trichoderma* sp.: manure with 1 : 400 (w/w) proportion and individual covering. The combination of *Trichoderma* sp. : manure with 1 : 400 (w/w) proportion and individual covering produced plant height and leaf length 8.5 cm and 4.4 cm, respectively, compared to the treatment without *Trichoderma* sp. addition that produced plantlets with 6.6 cm height and 3.4 cm leaf length. No significant interaction was shown between *Trichoderma* sp. proportions and covering types on leaf width, the number of leaves and root length parameters.

Keywords: acclimatization; banana; plant growth promoting fungi; *Trichoderma* sp.

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INTRODUCTION

Banana is one of the important parts of diet for many people in the world, including in Indonesia (Hallam, 1995; Wong et al., 2017; Murunde et al., 2018; FAO, 2019). This commodity is counted as the second most important fruit trade in the world after citrus (Hallam, 1995; Ortas et al., 2017). Banana are produced in many places of the world, such as Asia, Africa, Europe, Oceania, Central America and South America and among those, Asia is the largest banana production area in the world (Lahav, 1995; FAO, 2019). The banana plant from family Musaceae is known as the common fruit plant that are grown and cultivated in many areas in Indonesia. Banana var Barangan is one of commercial banana cultivars in Indonesia that is mainly produced in Deli Serdang and Simalungun Regencies (Nainggolan et al., 1998). Many farmers choose this banana cultivar to be cultivated because of its commercial value. However, this cultivar is categorized as susceptible to *Fusarium* wilt disease (Emilda et al., 2020).

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Banana is propagated by vegetative propagation methods, such as conventional propagation and micro propagation by tissue culture. The mainly used banana conventional propagation method is direct separation of banana tillers aiming at planting and killing the growing point of banana corm to induce axillary buds. The conventional vegetative propagation methods have several disadvantages, such as limited number of tillers, difficulties in transportation due to the size of tillers and the risk of infection by *Fusarium* (Ortas et al., 2017; Wong et al., 2017). Micro propagation can overcome several limitations found on conventional vegetative propagation. Micro propagation can produce mass banana seedlings with smaller amount of planting materials within short periods of time and smaller size of plantlets/planting materials and thus it will be easier to mass transported. This method can also reduce the chance of getting infected by diseases (Tenkouano et al., 2006; Chandra et al., 2010; Ortas et al., 2017; Kumar et al., 2019).

Acclimatization is one of the important processes in plant micro propagation before plantlets/planting materials are ready to be cultivated in the field (Pospóšilová et al., 1999; Hazarika, 2003; Thomas et al., 2010). The purpose of acclimatization process is to establish plant condition from *in vitro* to field condition (Robinson and Sáuco, 2009). There would be sudden changes of temperature, humidity and light conditions between *in vitro* and the field conditions. The sudden changes in environmental conditions promote abiotic and biotic stresses and even reduce the survival rate of the plantlets (Hazarika, 2003; Ortas et al., 2017; Wong et al., 2017; Shah et al., 2019). Furthermore, micro propagation of plants in the *in vitro* conditions may trigger the anatomy, physiology and morphology abnormalities of plantlets (Pospóšilová et al., 1999; Gutiarrez-Miceli et al., 2008; Chandra et al., 2010). Therefore, the acclimatization is needed to promote a normal growth of these plantlets and increase their survival rates.

During acclimatization, several treatments are used in order to prepare better adaptation of the plantlets in the new environmental condition. Hardening process, medium composition and covering types are different among plant species and varieties (Gutiarrez-Miceli et al., 2008; Winarto and Prama Yufdy, 2017; Shah et al., 2019). Covering the plant is an important step to reduce water loss during this process. Several plant species need transparent plastic and others use nylon mess covering types (Gutiarrez-Miceli et al., 2008; Winarto and Prama Yufdy, 2017). Some plants need single covering (one plant inside one cover) in order to survive, while the others can use mass covering.

Microbes play an important role in the acclimatization process of several plants (Pandey et al., 2000; Bag et al., 2001; Ortas et al., 2017; Shah et al., 2019). Dwarf Cavendish plantlets form mutualistic symbiosis with arbuscular mycorrhiza fungi to increase the plant growth and P-uptake (Ortas et al., 2017). Plantlets of *Cymbidium aloifolium* need to colonize with *Piriformospora indica* in order to increase their survival rate (Shah et al., 2019).

The application of several microbes, for example *Trichoderma* sp., are friendly to environment. Previous experiments showed that *Trichoderma* sp. could be a role model for sustainable agricultural practice (Singh et al., 2015; Al-Ani, 2018). These species are well known as plant growth promoting fungi (PGPF) and bio control agents for several soil borne diseases (Martínez-Medina et al., 2011; Barari, 2016; Khan et al., 2017; Castillo et al., 2019).

Trichoderma sp. can be used to increase the survival rate of plantlets during acclimatization in several plants. Gutiarrez-Miceli et al. (2008) stated that *Trichoderma* sp. increased the survival and growth rates of orchid plantlets *Guarianthe skinnerii*. Murunde et al. (2018) investigated the role of *Trichoderma* as plant growth promotor in Grand Naine and William Hybrid banana cultivars during weaning and potting stages. However, no information is obtained so far about the application of *Trichoderma* sp. during the acclimatization of banana plantlets cv. Barangan. The purpose of this study is to investigate the effect of *Trichoderma* sp. applications and covering types in the growth of banana plantlets cv. Barangan.

MATERIALS AND METHOD

Experimental site

The study was conducted in Aripan Experimental Station, Indonesian Tropical Fruit

Research Institute, from January to August 2019. The experimental site is located at 0°44'17" S and 100°37'20" E, approximately 425 meter above sea level in Solok Regency, West Sumatera Province, Indonesia. The means of annual temperature, rainfall and light time at this location are 24°C, 175 mm and 7 hours, respectively.

Experimental design

The study was arranged in a Completely Randomized Factorial Design with two factors and four replications. The first factor was covering type i.e. 1) individual covering and 2) mass covering. The second factor was the proportion of *Trichoderma* addition into manure, namely: a) manure without addition of *Trichoderma* sp. (control), b) *Trichoderma* sp. : manure = 1 : 400 (w/w), c) *Trichoderma* sp. : manure = 1 : 800 (w/w) and d) *Trichoderma* sp. : manure = 1 : 1,200 (w/w).

Plant material

Banana cv. Barangan is one commercial banana cultivar that origins from North Sumatera Province, with Deli Serdang and Simalungun Regency as the production centers of this banana (Nainggolan et al., 1998). This cultivar is included in triploid group (AAA) (Poerba et al., 2018) with potential production around 7 ton ha^{-1}.

Preparation of planting material

Banana plantlets cv. Barangan used in this study was obtained from tissue culture propagation. Six months before the acclimatization treatments, the explants obtained from banana corms were cultured. During the tissue culture period, the explants were sub-cultured four times in two different medium compositions. Media used in the initiation period were Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) + 4 ppm Benzyl Amino Purin (BAP) + 2 ppm Indole Acetic Acid (IAA). After that, these explants were sub-cultured in MS medium + 5 ppm BAP + 2 ppm IAA for three times. Banana plantlets with at least three leaflets were ready to be treated.

Preparation of *Trichoderma* mixture

Trichoderma isolate used in this study was obtained from Laboratory of Plant Protection, Indonesian Tropical Fruit Research Institute, Solok, West Sumatera. *Trichoderma* mixture was prepared two weeks before the acclimatization process started. Both of *Trichoderma* and manure were mixed together based on treatments and then kept in plastic bag for two weeks (Patent No. IDP 000037067, 2014). After two weeks, each combination was mixed with sterile soil and rice husk charcoal by comparison ratio of 2 : 6 : 1 (w/w/w). The combinations were collected and filled in each plastic pot.

The treatments process

Before the acclimatization process, the lid was removed from the bottle and the plantlets were separated from agar media into individuals and then washed in clean water. After that, all plantlets roots were removed neatly and dipped in fungicide for 15 minutes (Robinson and Sáuco, 2009). After that, plantlets were planted in plastic pots containing media with *Trichoderma* sp. proportion as stated previously and covered by transparent plastic cups or plastic sheets based on the treatments (individual covering or mass covering). Plantlets were maintained in same conditions during two weeks.

Data collection

The survival rates, plant height, leaf length, leaf width and number of leaves of plantlets were observed after two weeks in acclimatization process.

Statistical analysis

The data of survival rates, plant height, leaf length, leaf width and number of leaves were analyzed using software SPSS 17 software package (SPSS Inc., Chicago, Illinois, USA). Analysis of variance (ANOVA) was applied to test the experimental treatment effects, followed by Duncan New Multiple Range Test (DNMRT) at $p < 0.05$ if there were significant differences between the treatments.

RESULTS AND DISCUSSION

The survival rates, plant height, leaf length, leaf width and number of leaves of these plantlets were determined after fourteen days. This experiment resulted in similar survival rates of banana plantlets cv. Barangan in two factor treatments (Table 1). Both covering types and *Trichoderma* proportions did not significantly affect the survival rates of banana plantlets cv. Barangan. These treatments resulted in survival rates of banana plantlets var. Barangan, from 91.7% to 100%.

Table 1. The survival rates of banana plantlets cv. Barangan after two-week acclimatization on two covering types and four *Trichoderma* sp. proportions. The values in columns followed by the same letter are not significantly different at $p < 0.05$

Treatments	Survival rates (%)
Covering types	
- Individual covering	95.8±2.85 ns*)
- Mass covering	95.8±2.85
Trichoderma proportions	
- Manure without *Trichoderma* addition	100
- *Trichoderma* : manure = 1 : 400 (w/w)	95.8±4.17
- *Trichoderma* : manure = 1 : 800 (w/w)	91.7±5.54
- *Trichoderma* : manure = 1 : 1,200 (w/w)	95.8±4.17
Interaction	(-)

Note: (-) showing no interaction, where ns means that the treatments given did not give significantly different survival rates among all treatments based on ANOVA Test at $p < 0.05$; data are means of four replicates with standard error

Banana plantlets cv. Barangan at all treatments had higher survival rates. In most cases, the addition of bio control agent, such as *Trichoderma* sp., would provide better survival rates of plantlets during acclimatization (Gutiarrez-Miceli et al., 2008; Thomas et al., 2010). However, in this experiment, addition of *Trichoderma* sp. in medium composition did not affect the survival rates of banana plantlets. Probably, other factors, such as plantlet condition, environment condition and plantlet size, played their role in the survival rates of plantlets. Seed vigor and size determined the survival rates of papaya plantlets in acclimatization process (Damayanti et al., 2007).

In contrast with the survival rates, the treatments significantly affected the plant height, leaf length, leaf width, number of leaves and root length of banana plantlets (Figure 1, Table 2). The plantlet heights were significantly different between treatments at $p < 0.05$ (Figure 1). The highest plantlet was found in the interaction treatment between *Trichoderma* : manure = 1 : 800 (w/w) and individual covering (9.7 cm). However this result was not significantly different with the result shown in the treatment using *Trichoderma* sp. : manure = 1 : 400 (w/w) with individual covering and mass covering.

The application of *Trichoderma* sp. in appropriate proportion had a beneficial effect on plant growth in acclimatization process. This microorganism showed its role as plant growth promoter. Orchid seedlings, *Guarianthe skinnerii* that inoculated with *Trichoderma harzanium*, grew 3.6 times higher than in the control treatment (Gutiarrez-Miceli et al., 2008). Kushwaha et al. (2019) also reported that the Indian ginseng grew 15-30% higher on media with *Trichoderma* sp. compared to the control treatment.

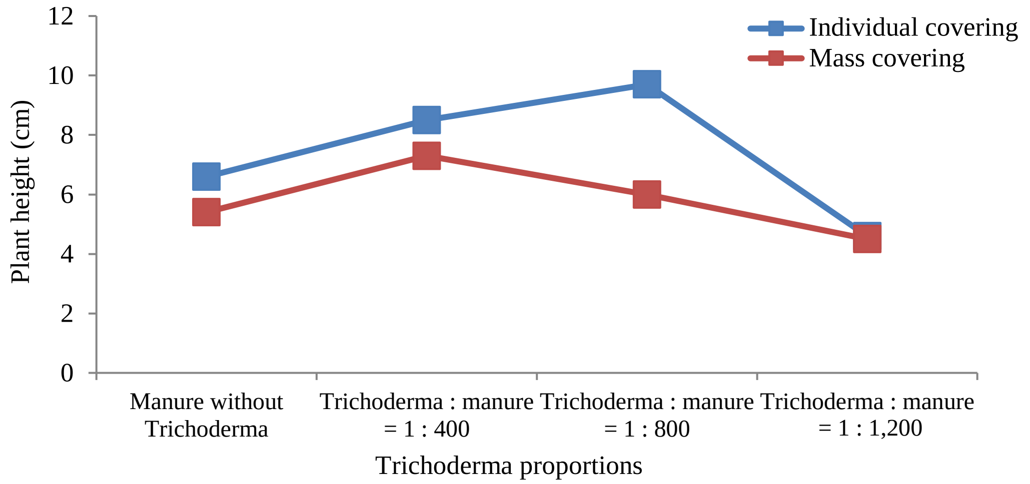

Figure 1. The data of plant height interaction between covering types with *Trichoderma* proportions

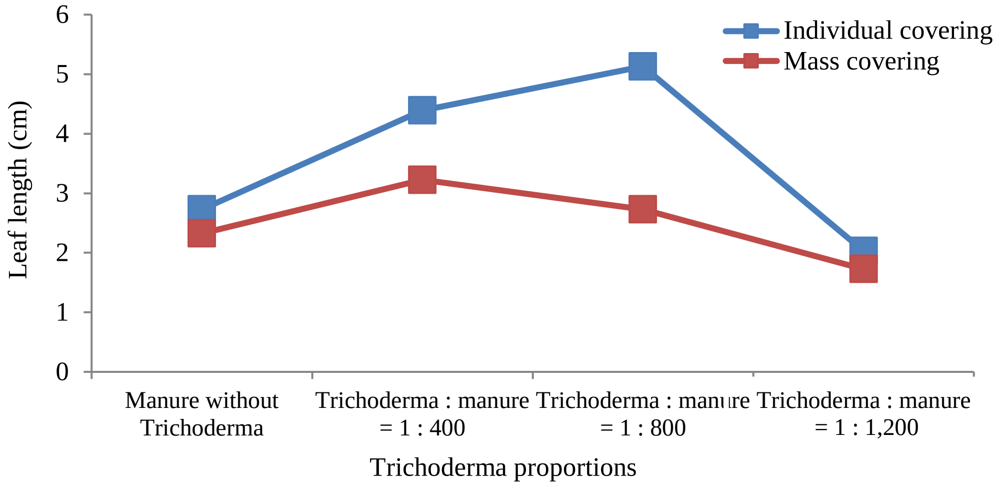

Figure 2. The data of interaction between leaf length and covering types with *Trichoderma* proportions

The interaction between addition of *Trichoderma* proportions and covering types significantly affect the leaf length of banana plantlets cv. Barangan at $p < 0.05$ (Figure 2). The best treatment for leaf length was found in the interaction between *Trichoderma* sp. : manure = 1 : 800 (w/w) and individual covering (5.1 cm). However, this result was not significantly different with the result shown in the treatment with *Trichoderma* sp. : manure = 1 : 400 (w/w) in individual covering. In contrast, the lower leaf lengths were found in several treatments, especially control treatment and *Trichoderma* sp. : manure = 1 : 1,200 (w/w) for both in individual covering and mass covering.

As shown in Table 2, the treatments given did not show interaction between the addition of *Trichoderma* proportion and covering type. Plantlets in individual covering significantly had wider leaves compared to mass covering, while the additional *Trichoderma* sp. : manure = 1 : 400 (w/w) resulted in wider leaves than the other treatments. However, this result did not significantly differ with the result indicated in the treatment with the addition of *Trichoderma* sp. : manure = 1 : 800 (w/w).

Table 2. The number of leaves, leaf width and root length of banana plantlets cv. Barangan after two-week acclimatization with two covering types and four *Trichoderma* sp. proportions. The values in columns followed by the same letter are not significantly different at $p < 0.05$

Treatments	Number of leaves	Leaf width (cm)	Root length (cm)
Covering types			
- Individual covering	3.1±0.18a	1.5±0.13a	3.8±0.46a
- Mass covering	2.4±0.17b	1.1±0.06b	3.2±0.24a
Trichoderma compositions			
- Manure without *Trichoderma* addition	2.7±0.15ab	1.2±0.07b	2.4±0.25b
- *Trichoderma* : manure = 1 : 400 (w/w)	3.3±0.21a	1.7±0.22a	4.4±0.37a
- *Trichoderma* : manure = 1 : 800 (w/w)	2.8±0.24ab	1.3±0.11ab	4.6±0.56a
- *Trichoderma* : manure = 1 : 1,200 (w/w)	2.1±0.30b	1.0±0.09b	2.6±0.33b
Interaction	(-)	(-)	(-)

Note: (-) showing no interaction, meaning that it is followed by different letter and shows significant difference according to DNMRT at $p < 0.05$; data are means of four replicates with standard error

Both leaf length and leaf width contribute to the total leaf area. Better results in leaf length and leaf width mean better leaf area. Leaf area had a significant part in plant growth and development due to leaf function in photosynthesis process (Gardner et al., 1991). Beside the genetic factor, environmental factors determined the leaf area of plant (Srihartanto and Indradewa, 2019). In this experiment, banana plantlets cv. Barangan were treated in different environmental conditions such as media mixtures and types of covering. Different environmental condition contributed to various leaf length and leaf width.

Table 2 shows the numbers of leaves of banana plantlets var. Barangan after the treatments. Treatment combinations of *Trichoderma* addition proportions and covering types significantly affected the numbers of leaves of banana plantlets; however, no interaction between those treatment factors were exposed. Similarly, as previous parameter, individual covering also resulted in better numbers of leaves compared to mass covering. The addition of *Trichoderma* : manure = 1 : 400 (w/w) produced the highest numbers of leaves compared to the other *Trichoderma* proportions.

In normal condition, the leaves of banana cv. Cavendish will grow within 8-10 days (Robinson and Sáuco, 2009). Gutiarrez-Miceli et al. (2008) reported orchid treated with *T. harzianum* produced higher number of leaves compared to that treated without *T. harzianum*. In line with this result, Murunde et al. (2018) reported that banana plants cv. Cavendish treated with *Trichoderma* yielded more leaves compared to the control.

Table 2 also presents that the covering types did not affect the root length of plantlets. In contrast, the proportions of *Trichoderma* addition significantly affected the root length of banana plantlets. The addition of *Trichoderma* sp. : manure = 1 : 400 (w/w) and *Trichoderma* sp. : manure = 1 : 800 (w/w) produced longer root than other treatments. The association between plant and beneficial microbes could support the nutrient uptake that resulted in longer root and higher number of lateral roots (Thomas et al., 2010). Even, Chandra et al. (2010) showed that the application of *T. harzianum* could trigger the root growth in *in vivo* condition.

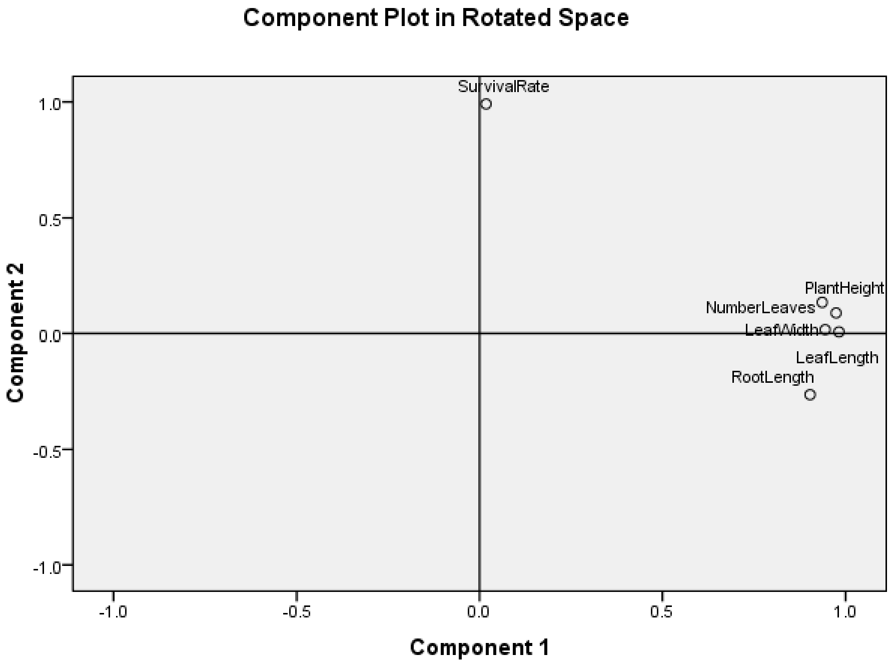

Figure 3. The Principal Component Analysis (PCA) test of six parameters of plants based on the covering types and *Trichoderma* proportions

The result from principal component analysis showed that the first principal component (PC1) accounted for 75% of the total variance, while the second principal component accounted for 18% an additional variance. PC1 represented the variation in five growth parameters of plant such as plant height, leaf length, leaf width, number of leaves and root length. PC2 only represented variation in survival rate of plant (Figure 3). The result showed that five growth parameters (plant height, leaf length, leaf width, number of leaves and root length) correlated each other. Based on leaf-height-seed (LHS) scheme, Westoby (1998) explained that three plant traits (leaf traits, plant height and seed mass) were fundamental for plant growth. These traits also correlated to and integrated with the other plant traits, for example root traits (Westoby, 1998; Laughlin et al., 2010).

The combination of both factors indeed affected the growth of banana plantlets. Several treatment combinations between the proportion of *Trichoderma* sp. addition and covering types provided better growth of banana plantlets cv. Barangan compared to the control treatment. The treatment combinations between the addition of *Trichoderma* sp. : manure = 1 : 400 (w/w) and *Trichoderma* sp. : manure = 1 : 800 (w/w) with individual covering type were the best combinations for the growth of plantlets. These combinations provided the best growth parameters comprising plant height, leaf length, leaf width and the number of leaves of banana plantlets cv. Barangan.

The application of *Trichoderma* improved the survival rates of plantlets and promoted better growth performance in other plant traits, such as stem diameter, fresh and dry weight of plants in the acclimatization process (Thomas et al., 2010; Murunde et al., 2018). The benefit of using this antagonistic fungus species in *in vitro* plantlets is due to the ability of this species to enhance nutrients and water uptake from growth media (Thomas et al., 2010; Colla et al., 2015). Thomas et al. (2010) also found that plants treated with *Trichoderma* produced higher contents of nitrogen and potassium compared to control plants.

In addition to those benefits, *Trichoderma* gave other benefits in agriculture. The application of *Trichoderma* could suppress the phyto-pathogenic infections in several plants (Benítez et al., 2004; El_Komy et al., 2015; Kushwaha et al., 2019). Banana cv. Barangan, which was used in this study is one of susceptible banana cultivars to *Fusarium* infections. Therefore, the application of *Trichoderma* since the acclimatization stage is expected to be able to prepare healthy plant of banana cv. Barangan before being planted in the field. Several *Trichoderma* strains are used as biofertilizers and myco-biocontrol agents (Mahfut et al., 2019; Silva et al., 2019). Some of them also help in plant growth and development in unfavorable environmental conditions (Benítez et al., 2004; Brotman et al., 2013; Contreras-Cornejo et al., 2014; Zhao et al., 2014).

CONCLUSIONS

The best treatment to enhance plantlet growth during the acclimatization process was the addition of *Trichoderma* sp. : manure with 1 : 400 (w/w) proportion and individual covering. The addition of *Trichoderma* sp. 1 : 400 produced similar plantlet performances with the treatments using higher *Trichoderma* sp. contents, but it used lower *Trichoderma* sp. proportion. Based on this research, we recommend the use of *Trichoderma* sp. : manure proportion = 1 : 400 (w/w) in combination with individual covering to improve banana plantlet growth during acclimatization.

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