

Effect of water content on conidia of *Trichoderma* spp., indole acetic acid content, electrical conductivity, and pH

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Abstract. Purwanto B, Sumadi, Nuraini A, Setiawati MR. 2022. Effect of water content on conidia of *Trichoderma* spp., indole acetic acid content, electrical conductivity, and pH. *Biodiversitas* 23: 2553-2560. *Trichoderma* sp. is a soil-borne saprophytic fungus that can increase plant resistance to combat drought stress. However, some species are unable to survive in a low water environment. Therefore, this research aimed to determine the effect of water content on different species of *Trichoderma* with respect to total conidial growth, IAA content, electrical conductivity (EC), and the media's pH. A factorial Completely Randomized Design (CRD) with 2 factors, namely the water content of the media consisting of 3 levels (5%, 25%, and 50%) and the inoculation of *Trichoderma* spp. which includes *T. harzianum*, *T. asperellum*, and *T. viride*. Furthermore, an experimental method was adopted by analyzing the total conidial growth, indole acetic acid content (IAA), electrical conductivity (EC), and acidity (pH) of the media. Incubation was carried out for 14 days, data were analyzed using one way ANOVA, and Duncan's Multiple Distance Test was applied to determine the parameters that have a significant effect. The results showed that the total conidia of *T. harzianum* at the lowest water content (5% of the dry weight of the media) showed an increase from 7th to 14th days after inoculation (DAI), the highest was 23.6%, followed by *T. asperellum* (12.6%) and *T. viride* (10.7%). Similarly, the total conidia of *Trichoderma* spp. positively correlated with the levels of IAA, EC, and pH of the media. This indicates that an increase in total conidia is followed by an increase in the IAA content, EC, and pH. The highest total of *T. harzianum* conidial growth at the lowest water content indicated that it had better resistance to drought stress than *T. asperellum* and *T. viride*.

Keywords: Conidia, indole acetic acid, inoculation, *Trichoderma*, water content

INTRODUCTION

The genus *Trichoderma* is a well known soil-borne saprophytic fungus that is widely distributed in all soil types (Mera-jul et al. 2012; Kalaivani et al. 2014; Wu et al. 2014). It is a good biocontrol agent as it suppressing the development of plant pathogens by producing antifungal enzymes such as chitinase and 1,3 gluconase (Harman 2006; Suada 2017). It also produces phytohormones such as auxins, which are required by plants (Haneefat et al. 2012) to increase their resistance to drought stress (Yu et al. 2014).

Trichoderma species are widely used in agriculture to stimulate the plant growth and to control different pathogens affecting crops, representing useful tools for sustainable food production. Applications of *Trichoderma* strains in agriculture to control fungal pathogens, nematodes and insects, the involved biocontrol mechanisms, efficacy and inoculation forms in greenhouse, field and post-harvest conditions (Ferreira et al. 2021; TariqJaveed et al. 2021).

An innovative technique that is increasingly being studied and applied in agriculture is the use of plant growth promoting microorganisms (bacteria and fungi) to induce

plant resistance to abiotic and biotic stresses (Poveda 2020). *Trichoderma* sp. is a rhizosphere fungus that is very important for agriculture and the environment, in inducing systemic resistance in plants (Kashyap et al. 2017; De Sousa et al. 2020). *Trichoderma harzianum* treatment during drought stress in rice plants can delay the drought up to 3-5 days (Bashyal et al. 2020). However, related to the ability of *Trichoderma* spp. to survive and thrive as well as its effect on the media under drought stress conditions has not been widely explored.

The influence of climate mitigation certainly has an impact on the rhizosphere conditions, specifically microorganisms that are beneficial for plant growth and development. The physiological processes of plants can be influenced by the growth regulators produced by certain microorganisms. *Trichoderma* a soil-borne saprophytic fungus helps to dissolve nutrients and release various compounds including phytohormones and some secondary metabolites that induce resistance responses to abiotic stresses (Shukla et al. 2012; Mastouri et al. 2010, 2012). Indole-3-acetic acid (IAA) (a type of auxin), one of the phytohormones produced by this species of *Trichoderma* promotes plant growth by increasing DNA and RNA

synthesis, as well as cell elongation through increased proton exchange (Aslamyiah 2002). However, its mechanism of action to promote plant growth is multivariate and influenced by the environment (Nieto-Jacobo et al. 2017).

Several studies have reported that the mechanism of action of *Trichoderma* spp. depends on humidity conditions around the plant roots. It grows well at 40-80% soil water content (Sudantha 2007) and its optimal value for enzyme activity range between 50-70% (Latifian et al. 2007). In extreme weather conditions (water content less than 40%), *Trichoderma* spp. may experience poor conidia growth, or perhaps fail to grow, thereby affecting the production of IAA.

Soil water content affects electrical conductivity (EC), which measures dissolved ions concentration. When there are many dissolved ions, the EC value will be higher (Suud et al. 2015). The high and low EC values affect cell wall activity, which degrades enzymes cellulase and glucanase by Binab T (commercial biocontrol product with an active strain of *Trichoderma polysporum* plus *T. harzianum*); enzyme production increases in line with the increase in EC (Khalil 2011). The degree of availability of water as a solvent affects EC level and the degree of acidity (pH).

The genus *Trichoderma* is sensitive and intolerant of high pH levels, as well as high salinity levels. In general, pH 6 is reported to be best for growth and sporulation of most *Trichoderma* isolates, while pH 8 showed a decrease in growth rate, colony diameter, and high sporulation compared to pH 4 (Ali et al. 2015). Therefore, this research aimed to obtain *Trichoderma* spp. that are more tolerant of low water content and also determine their effect on IAA content, EC, and pH of the media.

MATERIALS AND METHODS

Study area

The experimentation related to present study i.e. inoculation, incubation of test fungi, and counting of conidia was carried out at the Phytopathology and Biotechnology Laboratory of Pests and Diseases Department, Faculty of Agriculture, Padjadjaran University, West Java, Indonesia. The IAA content, EC, and pH were calculated at the Soil Biology Laboratory, Department of Soil Science and Land Resources, Faculty of Agriculture, Padjadjaran University, West Java, Indonesia from May to June 2019.

Experimental design

A factorial Completely Randomized Design (CRD) was adopted with two factors, namely *Trichoderma* spp. inoculation and water content of the media. The species of *Trichoderma* spp. used during this study were *T. harzianum* (i1), *T. asperellum* (i2), and *T. viride* (i3). The water content of the media used was 5% (k1), 25% (k2), and 50% (k3) of its dry weight, which obtained 9 combinations with 3 replications, hence, there were 27 experimental units.

Fungal material

This research used a pure *T. harzianum* culture obtained from the Phytopathology Laboratory, Department of

Phytopathology, Faculty of Agriculture, Padjadjaran University, West Java, Indonesia. Pure culture of *T. asperellum* and *T. viride* was also obtained from the Microbiology Laboratory of the School of Life Sciences and Technology, Bandung Institute of Technology, West Java, Indonesia. These three species belong to the genus *Trichoderma*, which have been used as biocontrol and plant growth stimulants (Harman et al. 2004).

Inoculation and incubation of *Trichoderma* spp. and conidial counting

The inoculation of the three species of *Trichoderma* (*T. harzianum*, *T. asperellum*, and *T. viride*) was carried out using potato dextrose agar (PDA) media in a petridish and then incubated in a relatively stable environment temperature conditions of 27 ± 2 °C for five days at the Phytopathology Laboratory, Faculty of Agriculture, Padjadjaran University, West Java, Indonesia.

The conidia of *Trichoderma* spp. were counted after 5 days of incubation and the sample was examined using a Marienfeld Superior brand hemocytometer from Germany. For this a dilution was carried out first (Shukla et al. 2012) by taking 1 mL of the suspension solution using a micrometer pipette, then diluted with 9 mL of sterile distilled water in a 20 mL beaker. The dilution was stirred evenly with a vortex mixer resulting in a suspension of 10^{-1} . This process was continued until 10^{-6} obtained and 1 ml of the suspension was observed with the help of hemocytometer for counting of conidia. It was subsequently observed using a microscope with 400 x magnification.

Effects on total conidia, Indole Acetic Acid (IAA) content, electrical conductivity (EC), and the degree of acidity (pH)

Each species was grown on 50 g sterile media (soil plus 10% cow dung and 10% rice husk biochar) in glass bottles containing different sterile distilled water according to the media's water content. Glass bottles were previously sterilized by autoclaving at 15 psi for 20 minutes and then each *Trichoderma* spp. was inoculated with 2 ml of conidia suspension (10^6 conidia mL⁻¹). It was then incubated at 26 ± 2 °C lasted for 14 days. Glass bottles were weighed periodically, and when the water content decreases, it is refilled with sterile distilled water according to the treatment.

Observation

The response variables observed were the total conidia in each *Trichoderma* spp., Indole Acetic Acid (IAA) content, electrical conductivity (EC), and the degree of acidity (pH).

Total conidia of *Trichoderma* spp.

Counting was carried out on 7th and 14th days after inoculation (DAI) using 1 g of media air-dry weight (g⁻¹ substrate air-dry weight), then serial dilutions were carried out (Shukla et al. 2012), and calculated using a hemocytometer. Conidia/spore calculation with the formula:

$$K = \frac{t \times d}{n \times 0.25} \times 10^6$$

Where:

K : Number of conidia mL⁻¹ solvent
 t : Number of conidia in all sample boxes
 d : dilution factor
 n : The sum of all calculated sample boxes
 0.25 : Correction factor

Indole Acetic Acid (IAA) content

The concentration of IAA was measured using the modified Patten and Glick (2002) method for 14th days after inoculation (DAI) of test fungi by using a spectrophotometer at a wavelength of 520 nm. The IAA concentration was calculated after being compared with the absorbance of its standard solution. The result obtained from the spectrophotometer shows the relationship between the IAA standard solution (x) and its absorbance (y), using the equation below:

$$Y = a + bx$$

Where :

a : intercept
 b : Slope (Regression coefficient)
 Y : Absorbance
 x : Concentration

Degree of acidity (pH) medium

The media's pH was measured using the Orion brand pH meter model 210A on the 14th days after inoculation (DAI).

Electrical conductivity (EC)

EC data was measured using the Hanna Instrument (HI 98128) on 14th days after inoculation (DAI).

Data analysis

Analysis of Variance (ANOVA) from SPSS software version 25 was adopted for data analysis and continued with the DMRT (Duncan Multiple Range Test) to test the parameters that had a significant effect at 5% level.

RESULTS AND DISCUSSION

Effect on total conidia *Trichoderma* spp. on 7th days after inoculation (DAI)

In general, increasing the water content of the media will increase the number of conidia *Trichoderma* spp. The interaction treatment of water content 50% dry weight of media with *T. harzianum* (k3i1) resulted in the highest total conidia (9.83 x 10⁸ conidia mL⁻¹) and was significantly different from *Trichoderma* spp. another at the same water content. The interaction of *T. harzianum* with increasing water content showed a significantly different effect on the total number of conidia. The highest total conidia at the lowest moisture content (5% of media dry weight) were produced by *T. harzianum*, although not significant with *T. asperellum* and *T. viride* (Table 1).

The growth of the total conidia produced by *Trichoderma* spp. depends on the water content of the given medium. The total conidia produced by *T. harzianum* was more than *T. asperellum* and *T. viride*, indicating that it has better adaptability at low water content.

Table 1. Total conidia of *Trichoderma* spp. on 7th days after inoculation (DAI) at various water content

Medium water content	Total conidia (10 ⁸ conidia mL ⁻¹)		
	i1 (<i>T. harzianum</i>)	i2 (<i>T. asperellum</i>)	i3 (<i>T. viride</i>)
k1 (5% media dry weight)	5.92 c A	5.83 b A	5.58 a A
k2 (25% media dry weight)	7.00 b A	6.83 a A	5.42 a B
k3 (50% media dry weight)	9.83 a A	7.00 a B	5.83 a C

Note: Numbers followed by lowercase letters in the same column (read vertically) and capital letters in the same row (read horizontally) were not significantly different based on DMRT at the 5% level

Table 2. Total conidia of *Trichoderma* spp. on 14th days after inoculation (DAI) at various water contents

Medium water content	Total conidia (10 ⁸ konidia mL ⁻¹)		
	i1 (<i>T. harzianum</i>)	i2 (<i>T. asperellum</i>)	i3 (<i>T. T. viride</i>)
k1 (5% media dry weight)	7.75 b A	6.67 a B	6.25 a B
k2 (25 % media dry weight)	8.33 b A	7.92 a A	6.25 a B
k3 (50% media dry weight)	11.25 a A	7.92 a B	6.75 a C

Note: Numbers followed by lowercase letters in the same column (read vertically) and capital letters in the same row (read horizontally) were not significantly different based on DMRT at the 5% level.

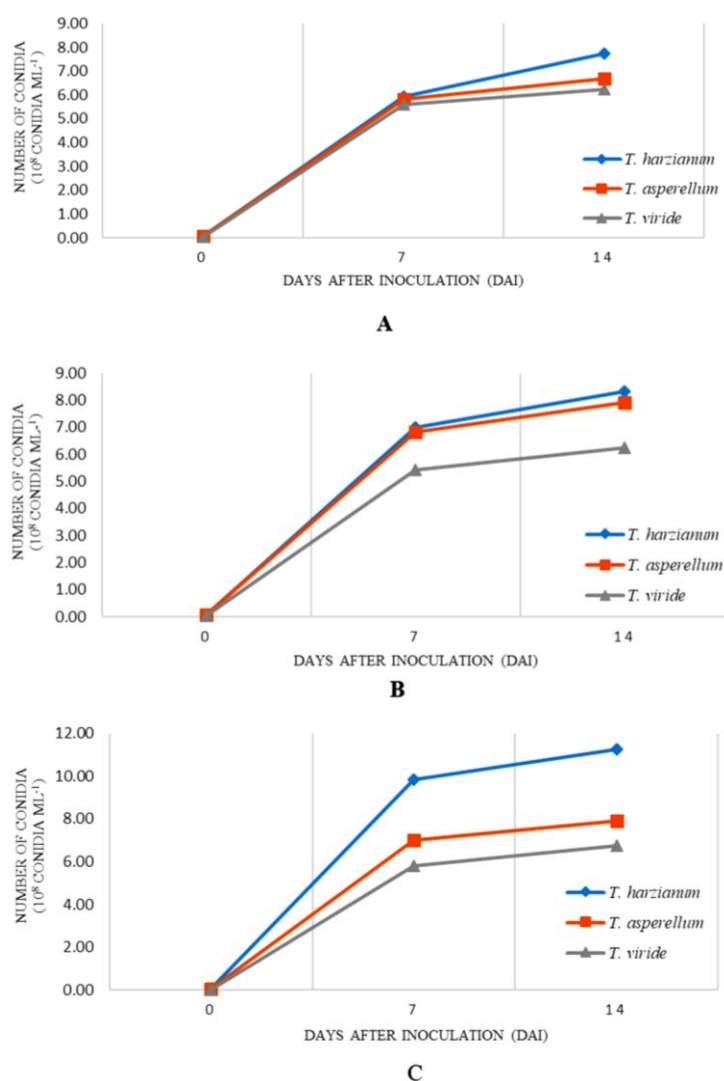


Figure 1. The growth dynamics of *Trichoderma* spp. total conidia on 7th to 14th days after inoculation (DAI) at various media with a water content of 5% (A), 25% (B), and 50% (C)

Total conidia *Trichoderma* spp. on 14th days after inoculation (DAI)

Increasing the water content of the media significantly increased the total *Trichoderma* spp. conidia on 14th days after inoculation (DAI). Furthermore, Duncan's test showed that the interaction of 50% water content of the media's dry weight with *T. harzianum* (k3i1) resulted in the highest total conidia (11.25×10^8 conidia mL⁻¹) and was significantly different from other species. The treatment of water content in the same type of *Trichoderma* also showed significant differences. *T. harzianum* produced the most total conidia at the lowest content (5% of the medium's dry weight) on 14th days after inoculation (DAI), which significantly differs from *T. asperellum* and *T. viride* (Table 2).

In general, the total conidia growth produced by *T. harzianum* is the highest compared to *T. asperellum* and *T. viride*, both on 7th to 14th days after inoculation (DAI). The

growth dynamics of *Trichoderma* spp. total conidia are shown in Figure 1.

Trichoderma harzianum produced higher conidia growth than *T. asperellum* and *T. viride*, both at 5%, 25%, and 50% water content of the media dry weight (Figure 1).

Effect of water content with *Trichoderma* spp. to the IAA content

The results of Duncan's test showed that giving water content had a significant effect on IAA levels while giving *Trichoderma* spp. inoculation had no significant effect. The highest level of IAA (1.30 ppm) was produced by giving 50% water content of the media dry weight, while the lowest (0.69 ppm) was at 5%. The inoculation of *T. harzianum* and *T. viride* resulted in the highest (1.01 ppm) and lowest (0.91) levels of IAA, respectively. However, the inoculation of the three types of *Trichoderma* spp. has no significant effect on IAA levels (Table 3).

Effect of water content with *Trichoderma* spp. against the electrical conductivity (EC) media

The ANOVA results showed that the water content interaction with *Trichoderma* spp. had a significant effect on the EC of the media. The highest EC value of 1760.33 S cm⁻¹ was produced by *T. viride* at 5% water content of the media's dry weight, which was significantly different from the value produced by *T. harzianum* and *T. asperellum* (Table 4).

The EC value produced by *T. harzianum* and *T. asperellum* tended to increase in line with the increase in water content, while *T. viride* tended to decrease with the increase in the water content (Figure 2).

Effect of water content with *Trichoderma* spp. on pH of the medium

The ANOVA results showed that the water content interaction with *Trichoderma* spp. has no significant effect on the pH of the media. Furthermore, Duncan's test showed that the water content treatment significantly affects the pH of the media, while the inoculation treatment of *Trichoderma* spp. has no real effect. This shows that the water content of the media has a more significant effect on the pH than the inoculation factor of *Trichoderma* spp. In addition, the lowest pH value produced by *T. harzianum* was 7.35, which was still within the neutral pH range (Table 5).

Figure 3 shows that the pH increased in line with the increase in the water content. At 5% water content of the media dry weight, an increase in the pH value of 2.06% (25% water content) and 3.52% (50% water content) was observed.

Correlation between total number of conidia *Trichoderma* spp. with levels of IAA, EC, and pH

Total number of conidia *Trichoderma* spp. significantly correlated with IAA levels. However, there was no significant correlation between EC values and media pH. Furthermore, the correlation between the total conidia and the levels of IAA, EC, and the pH was positive, indicating that an increase in total conidia is followed by an increase in IAA levels, EC, and the pH. Similarly, a decrease is followed by a decrease in IAA levels, EC, and pH medium (Table 6).

Discussion

The higher total conidia of *T. harzianum* than *T. asperellum* and *T. viride* indicated that it has better adaptability at low water content. This is consistent with

the report of Rossi-Rodrigues et al. (2009) that among *Trichoderma* species, the growth profile of *T. harzianum* was faster than *T. viride* and *Trichoderma longibrachiatum* in various nutritional sources and physical conditions.

Table 5. Effect of media water content with *Trichoderma* spp. on pH of the medium

Treatment	pH
Water content	
k1 (5% media dry weight)	7.25 c
k2 (25% media dry weight)	7.42 b
k3 (50% media dry weight)	7.54 a
<i>Trichoderma</i> spp.	
i1 (<i>T. harzianum</i>)	7.35 a
i2 (<i>T. asperellum</i>)	7.43 a
i3 (<i>T. viride</i>)	7.43 a

Note: Numbers followed by the same lowercase letters are not significantly different based on DMRT at the 5% level.

Table 6. Correlation between total number of conidia with levels of IAA, EC, and pH

Parameter	IAA level	EC	pH
Total conidia 14 HSI	0.694*	0.408	0.210
IAA level		0.240	0.605
EC			0.015

Note: * The correlation is significant at 0.05 level.

Table 3. Effect of media water content with *Trichoderma* spp. to the level of IAA

Treatment	IAA level (ppm)
Water content	
k1 (5% media dry weight)	0.69 b
k2 (25% media dry weight)	0.85 ab
k3 (50% media dry weight)	1.30 a
<i>Trichoderma</i> spp.	
i1 (<i>T. harzianum</i>)	1.01 a
i2 (<i>T. asperellum</i>)	0.92 a
i3 (<i>T. viride</i>)	0.91 a

Note: Numbers followed by the same lowercase letter are not significantly different based on DMRT at the 5% level.

Table 4. Effect of interaction of water content with *Trichoderma* spp. against the Electrical Conductivity (EC) of the media

Water content (%)	Electrical conductivity (μS cm ⁻¹)		
	i1 (<i>T. harzianum</i>)	i2 (<i>T. asperellum</i>)	i3 (<i>T. viride</i>)
k1 (5% media dry weight)	1259.67 b B	1160.67 a B	1760.33 a A
k2 (25% media dry weight)	1403.00 b A	1567.00 a A	1413.33 b A
k3 (50% media dry weight)	1715.33 a A	1546.33 a AB	1140.33 c B

Note: Numbers followed by the same lowercase letter (vertical direction) and the same uppercase letter (horizontal direction) were not significantly different based on DMRT at the 5% level.

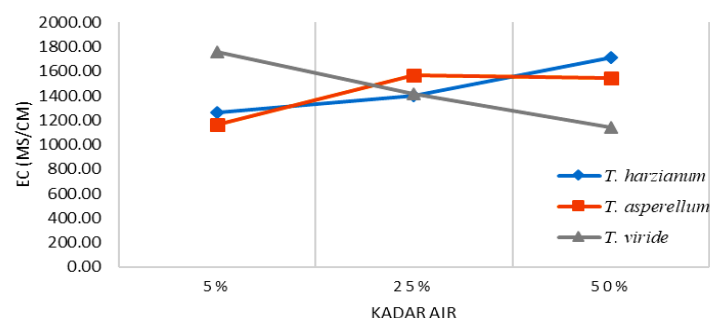


Figure 2. Effect of the media water content and *Trichoderma* spp. against EC

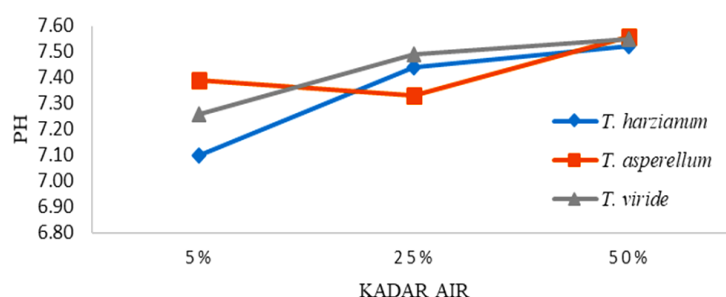


Figure 3. pH of the media at several levels of water content and *Trichoderma* spp.

Increasing the media water content has a significant effect on the increase in the total conidia produced by *T. harzianum* and *T. asperellum*, except for *T. viride*. Despite the lack of significant effect, *T. viride* total conidia tended to increase with increasing water content. Furthermore, the optimum water content required by *Trichoderma* spp. to produce the most spores is $64.41 \pm 0.08\%$ and increasing humidity to $73.13 \pm 0.31\%$ reduce the total spores produced (Cavalcante et al. 2008). According to Sudantha (2007), the fungus *Trichoderma* spp. can grow well in soil water content ranging from 40% to 80%. The water content of the media affects the activity of *Trichoderma* cellulose and it is optimal for enzyme activity at the range of 50% - 70%. The addition of water above 75% will cause a decrease in the activity value, thereby inhibiting conidia's growth (Latifian et al. 2007).

Based on the overall results, there is a relationship between the total conidia, IAA content, EC values, and pH of the media. The best total conidia at low water content (5% and 25% media dry weight) were produced by *T. harzianum*, followed by *T. asperellum* and *T. viride*, respectively. This implies that higher the total conidia produced by *T. harzianum*, higher the IAA content as compared to those produced by *T. asperellum* and *T. viride*. This is because one of the factors that affect IAA content is conidia's growth rate. Other factors include supernatant culture, tryptophan concentration, carbon source, agitation, dissolved oxygen concentration, and incubation time (Bose et al. 2013).

The overall IAA production from *T. harzianum*, *T. asperellum*, and *T. viride* were still in a low range compared to other rhizosphere fungi which are in the range of 4,329 ppm (Subowo 2013) and the highest of 19.6 ppm (Lasmini 2016). According to Patten and Glick (2002), each type of microbe can produce a different IAA concentration, which is influenced by the type of microbe, total nutrients, incubation time, growth rate, the varying concentration, and the ability to convert tryptophan in the media into IAA. Furthermore, natural sources can be produced from a cow dung fertilizer, although in a very small amount. The media used in this research was soil plus 10% manure, and 10% rice husk biochar, since cow dung fertilizer is used as a source of nutrition and as a tryptophan precursor. Kresnawaty et al. (2008) stated that cow dung is a source of tryptophan and can be purified, however, its production of IAA is lower than synthetic tryptophan because it has low levels of purity.

Indole acetic acid (IAA) and other secondary metabolites produced by soil microorganisms, such as gliotoxin produced by *Trichoderma virens* are inhibitory to various fungi and phytopathogenic bacteria, their production is strongly influenced by soil moisture, soil microbial community and pH conditions (Jayalakshmi et al. 2021).

These results indicated that the water content and *Trichoderma* spp. have no significant effect on EC value. Although it had no significant effect, the increase in the water content tended to further increase the EC value. Furthermore, this value was used to express dissolved salts

for soil salinity (Shirokova et al. 2000). In this research, the EC values obtained are in the salinity range of 1140.33 - 1760.33 S cm⁻¹ and are included in the non-salinity level or class 0 (Muliawana et al. 2016). *Trichoderma harzianum*, *T. asperellum*, and *T. viride* responded well to the values in this range, hence, they did not interfere with the growth of their conidia.

The EC value of the media tends to increase along with the increase in the water content of the media, presumably because the water content in the soil will help the potential nutrient levels to dissolve and hydrolyze, thereby forming ions and cations. This is consistent with the reports of Rogero et al. (2013) that the water content treatment will increase the EC value on loamy soil to the highest at 48.9% water content, which tends to decrease again at > 48.9%. Meanwhile, loamy soils contain equal amounts of sand, silt, and clay. When the water content in the soil is too saturated and the macropores of the soil are filled with water, the electrical conductivity value due to saturated water content dominates the EC measurement rather than the electrical conductivity value due to hydrolyzed nutrients ion (Seladji et al. 2010).

The results verified that increasing the water content resulted in a tendency for the pH of the media to increase because it was suspected that there were more hydroxide ions (OH⁻) than hydrogen ions (H⁺) dissolved in water. On the other hand, the decrease in water content is also followed by a decrease in the pH level. This is consistent with the report of Banjarnahor et al. (2018) that a decrease in soil water content is followed by a decrease in soil pH and conversely an increase is also followed by an increase in soil pH value.

The increase in the pH to alkaline is less favored by *Trichoderma* spp. as evident from the results where an increase in the pH is followed by a decrease in the total conidia. This is consistent with the statement of Limon et al. (2004), that *Trichoderma* spp. prefers an acidic pH ranging from 4.6 to 6.8 for best growth. Their growth rates were reported to be no higher at pH 4.0 or less (Verdin et al. 2004; Singh et al. 2014). Furthermore, at pH 8 (alkaline), *T. harzianum*, *T. asperellum*, and *T. viride* were still growing, which is consistent with Singh et al. (2003), that *T. harzianum* can still grow at pH 8 even though its biomass product is lower than at pH 7.5.

The correlation between the total conidia and IAA content, EC, and pH was positive. This indicates that an increase in total conidia is followed by an increase in the IAA content, EC, and the pH of the medium. Similarly, a decrease in the total conidia is followed by a decrease in the IAA content, EC, and pH. The increase in the total conidia also increased *Trichoderma*'s activity in helping to remove solutes, thereby increasing the EC value. The activity of *Trichoderma* is certainly increased at a suitable pH, hence, it will produce higher IAA content in the end.

Conclusively, increasing water content significantly increases the total conidia, IAA content, EC, and pH of the media. Also, the total conidia's growth was positively correlated with IAA content, EC, and pH. At a moisture content of 5% of the media dry weight, the growth of *T. harzianum* total conidia was higher than that of *T.*

asperellum and *T. viride*, hence it was declared to be more tolerant at low water content.

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