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EFFECTS OF SHADE AND *TRICHODERMA* ON VEGETATIVE GROWTH RESPONSE OF UPLAND RICE TO ACID SOIL

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ABSTRACT

Trichoderma fungus isolates have begun to be recognized as biofertilizers, which are expected to not only play a role in breaking down organic material that produces nutrients for plants, but also help plants to live normally in acidic soil conditions and dry land with low light intensity. The initial growth period is a critical phase for upland rice in facing environmental stress including low light intensity and soil acidity. The aim of this research was to determine the effects of providing 60% paranet shading and *Trichoderma* biological fertilizer on the vegetative growth response of upland rice leaves on acid soil. The experimental treatments were arranged in a split plot design. The primary plot is comprised of two categories: "without shade" and "shade of 60%". The subplots are *Trichoderma* biological pupils consisting of: without *Trichoderma* or control, *Trichoderma* isolate Tc-Jjr-02 and *Trichoderma* isolate Tc-Jro-02. The treatment was repeated 4 times to obtain a total of 24 experimental units. Weekly observations were made of plant height growth and number of tillers, as well as, the total amount of chlorophyll and the total amount of carotenoids at the end of the vegetative phase. Observational data were analysed using analysis of variance at the 5% level to determine the effect of treatment on the growth response of upland rice. To determine the differences between treatments, an Honest Significant Difference (HSD) test was performed at the 5% level. The results showed that shade, biofertilizer and interaction had no significant effect on plant height, but a very significant effect on number of tillers. Application of *Trichoderma* biofertilizer without shade showed most tillers and was able to help upland rice plants overcome acid soil stress in the vegetative phase. The shade of 60% can increase 29.8% total chlorophyll and 40% total carotenoids of upland rice leaves. The interaction between shade and *Trichoderma* biological fertilizer has made a significant impact on the number of tillers in treatments 21 and 56 days after planting.

Key words: Carotenoid, chlorophyll, shade, *Trichoderma* biological fertilizer, upland rice

INTRODUCTION

Rice is a very strategic commodity in Indonesia, so efforts to maintain its availability continue to be developed. However, to secure food stocks, imports are often carried out. The ability of existing land resources is actually able to meet consumption needs, but natural disasters and other production disruptions can threaten food stocks. Environmental factors such as soil, climate, and intruding organisms are limiting rice crop production in Indonesia [1]. The development of upland rice planting on dry land actually has a large and reliable potential for efforts to improve food security [2]. Some of these dry lands are forestry and plantation lands with distinctive characteristics, namely; low soil fertility [3], low pH (acid soil), drought stress [4], and shade by tree canopy, especially when used as an intercropping system [5]. Soil with a degree of acidity outside the pH range of 6-7 will inhibit and reduce plant productivity [6]. Dry land that has potential for developing upland rice is forest and plantation areas. In this case, rice can be intercropped with perennials as staple crops. However, the challenge is that the main crop canopy has the potential to suppress rice growth. In the Sengon forest, the shade intensity is 10%-80% [7], while in the mahogany forest it is between 36.50%-84.38% [8] or an average of 60% which makes it possible to produce upland rice. Barriers in the form of low light intensity greatly affects the quality and quantity of light waves which fall to the surface of the leaf and the water balance that passes through the stoma opening [9, 10]. These conditions will affect the response of plants in producing devices for the process of photosynthesis and photosynthates produced [11]. Chlorophyll and carotenoid are photosynthetic pigments which are important tools in the process of photosynthesis that play a role in absorbing light radiation. Interference in absorption of light intensity is thought to affect the quantity of chlorophyll and carotenoid molecules in leaf cells.

Many rice varieties are less tolerant of acidic soils. To overcome the threat of soil acidity stress, the use of symbionts from effective microbial groups that normally grow well in the rhizosphere is highly considered. Provision of organic material in dry land will improve soil fertility, but enriching it by providing effective microorganisms, or applied as biological fertilizer will be beneficial for efforts to increase fertility of dry land. Effective soil microorganisms will help improve soil physical properties through the formation of soil microaggregates [12] which are very important for the supply of plant nutrients. *Trichoderma* fungi are one of the symbionts that can live in a rhizosphere of plants that are able to live and carry out their role in acid dry soils [13].

Trichoderma apart from playing a role in degrading organic matter and [14, 15], also produces β-1,3-glucanase, cellulase, and peroxidase enzymes [16], thus providing a positive effect on providing nutrients to plants [17]. *T. harzianum* in addition to increasing dryland crop yields, also increases plant resistance to disease and protects plants from pathogens [18, 19]. *T. harzianum* produces the enzyme chitinase which can degrade chitin compounds from the pathogenic fungal cell wall [20].

With its significant role [21], the application of *Trichoderma* fungi isolates as biological fertilizer is expected to not only play a role in overhauling the organic material that produces nutrients for plants, but also helps plants to be able to live normally under acidic soil conditions and dry land with low light intensity. This research aimed to determine the effect of shading and the application of *Trichoderma* biofertilizer on the vegetative growth response as well as total chlorophyll and total carotenoids of upland rice plant leaves.

MATERIALS AND METHODS

Place and time of research

In this study there were two stages of activity, namely; the application of shade and biological fertilizer experiments in open spaces in the Green House (5 m asl), followed by analysis of total chlorophyll and leaf carotenoids at the Chemical Analysis Laboratory of Universitas Muhammadiyah Sidoarjo in Gelam Village, Candi District, Sidoarjo Regency. The experiment was conducted in the dry season from March to July 2022.

Shade and biofertilizer application test

In this experiment an entisol soil with a pH of 4.8 was used as a growing medium obtained from the village of Purwojati, Ngoro District, Mojokerto Regency, East Java. Before being used, the soil was sterilized in an autoclave at a temperature of 121^{o}C, pressure of 1 atm for 30 minutes. Sterile soil was put into a polybag. The polybag capacity was 5 kg.

Trichoderma isolates Tc-Jjr-02 and Tc-Jro-01, as active ingredients of biological fertilizer were obtained from the collection of Agricultural Microbiology Laboratory of Muhammadiyah University, Sidoarjo. Both isolates were grown on PDA-m media for 10 days. The culture of incubation of *Trichoderma* isolates was mashed using a mixer and diluted to become a suspension. To find out the isolated population in a certain volume unit, a series of dilutions of up to 10^8 was carried out. As much as 1 ml suspension was taken using a sterile syringe and sprayed onto the inner surface of a petri dish containing PDA-m media. The appearance of small green spots after three days of incubation indicates the number of colony-forming units which represents the population density per ml of suspension. Based on the results of the test population by means of the dilution, the sterile distillation or addition of water was carried out in order to obtain an average population of the two types of isolates equal to each ml of suspension which was $0.5x10^8$ cfu/ml. Each suspension was then poured into sterile compost and the density was adjusted to $5x10^7$ cfu/g of compost. In each polybag containing 5 kg of soil, 200 grams of compost were mixed

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evenly; thus, the population of each *Trichoderma* isolate in the growing medium in each polybag was $2x10^6$ cfu/gr.

Situpatenggang variety rice seeds were immersed in 50% alcohol solution for 3 seconds to kill unwanted organisms on the surface of the seeds, then rinsed with distilled water three times and drained. This sterile surface seed was placed into a nursery tray that contains moist soil and was incubated for 10 days to germinate. Into each polybag were placed two rice sprouts, piled with soil and gently stained with water until the medium soil grew wet. Furthermore, watering was done every morning and evening with an average volume of 500 ml per polybag or until the soil was wet.

Polybags containing plants and growing medium were given biological fertilizers, namely isolates of *Trichoderma* Tc-Jjr-02 and Tc-Jro-01 and without *Trichoderma* (control), placed in two different conditions namely shading conditions (paranet shading 60%) and without shade. The experimental treatments were arranged in a split plot design. The main plot is in the form of shade consisting of: without shade (S0) and shade using 60% paranet (S1). The subplots were *Trichoderma* biological pupils consisting of: without *Trichoderma* or control (T0), *Trichoderma* isolate Tc-Jjr-02 (T1) and *Trichoderma* isolate Tc-Jro-02 (T2). Each of the six combination treatments were repeated four times to obtain a total of 24 experimental units.

Every week at 14 to 56 days after application (DAA), plant height and number of tillers were observed. Measurement of sunlight intensity (lux meter) and air temperature above the canopy was done every day around 12:00 a.m. Each of the measurement data was calculated on average weekly. The pH of the soil growing medium per polybag was measured every week using a pH meter.

Analysis of total chlorophyll and carotenoids

Analysis of total chlorophyll and total carotenoids obtained from leaf extracts of upland rice plants was carried out at the end of observation (56 DAA) by means of Kurniawan, Izzati, and Nurchayati (2010), which were modified by determining the levels of the two kinds of leaf pigments using a UV-Vis spectrophotometer. A 0.1 g of plant leaves were crushed in a mortar and 10 ml of acetone was added. The extract was filtered with Whatman 41 paper. The filtrate was then measured for absorbance at 480, 645, and 663 nm. Chlorophyll content was calculated using formula (1), while the rubberinoid content was calculated by formula (2) below:
Total chlorophyll (mg/g leaf tissue weight):

$$8.02 \times A663 + 20.2 \times A645 \times 10^{-1} \quad (1)$$

Carotenoids (μmol/g leaf tissueweight):

$$((A480 + 0.114 \times A663 - 0.638 \times A645) \times V \times 10^3) / (112.5 \times 0.1 \times 10) \quad (2)$$

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With the provisions: A663, A645 and A480, are absorbance values at 663, 645 and 480 nm, respectively; V was the extract volume.

Data analysis
Observation result on plant height (cm) and number of tillers each week from 21 to 56 DAA and total chlorophyll (mg/g) and total carotenoids (µmol/g) were analyzed using Analysis of Variance (ANOVA) at 5% level to determine the effect of treatment on upland rice growth response. To find out the differences between treatments, the Honestly Significant Difference (HSD) test was performed at a level of 5%.

RESULTS AND DISCUSSION

Average light and temperature intensity
Based on the results of daily observations of light intensity and air temperature above the canopy both under paranet 60% or without paranet, the weekly average was calculated and presented in Table 1.

Plant height
The results of the analysis of variance on plant height from 14 to 56 DAA of *Trichoderma* biological fertilizer and shade showed that the interaction between biological fertilizer was not significant ($p>0,05$). Likewise, the inter-treatment both on the shade factor and the biofertilizer factors did not show any influence of differences.

The average height of plants in the shade factor treatment is shown in Figure 1, whereas the treatment of biological fertilizer factors is shown in Figure 2.

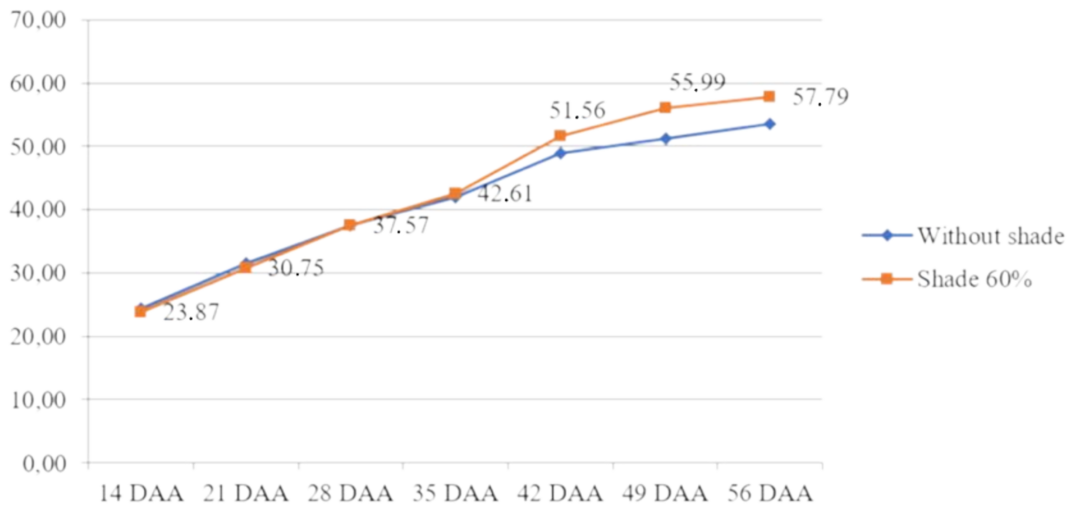

Figure 1: The mean of upland rice plant height in response to shade at 14-56 DAA (cm)

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As shown in Figure 1, it appears that the growth curves from 14 to 35 DAA coincide. Starting at 42 DAA, the growth of upland rice under a shade of 60% appears to be higher than the control until the end of the observation of the vegetative phase or entering the generative phase. The performance of these two biological age isolates showed no difference as indicated by the growth curves of upland rice plants in both treatments almost coinciding between 14 and 56 DAA (Figure 2).

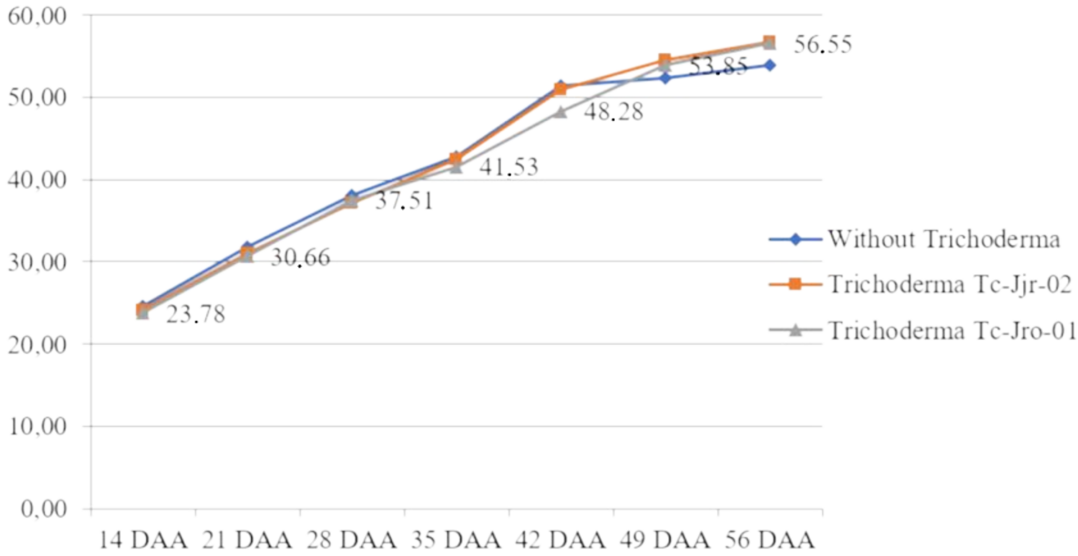

Figure 2: **The mean of upland rice plant height in response to *Trichoderma* biological fertilizer in 14-56 DAA**

Number of tillers

The results of the analysis of the number of upland rice seedlings (14-56 DAA) showed that there was a real interaction between shade and biological fertilizer that was not significant ($p>0.05$) at 28 and 56 DAA. Meanwhile, both shade and biological fertilizer had a significant effect ($p<0.05$) on plant response in the form of the number of tillers produced from 14 to 56 DAA.

The mean number of upland rice tillers in response to a combination of shade and biological fertilizer treatments is shown in Table 2. Meanwhile, Figure 3 shows the growth in the number of offspring from 14 DAA to 56 DAA in all treatment combinations.

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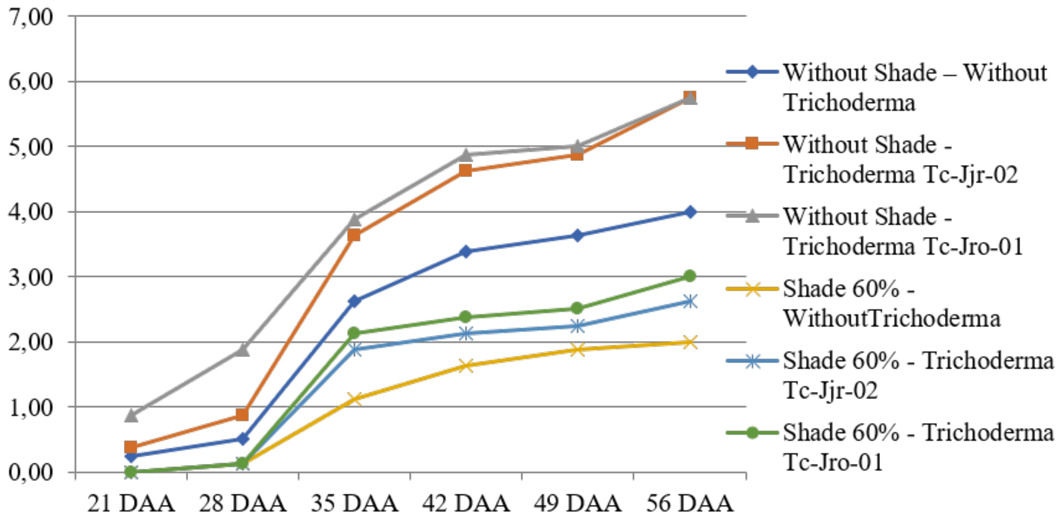

Figure 3: **The mean number of tillers as affected by a combination of shade and *Trichoderma* bio-fertilizers at 21-56 DAA**

Total chlorophyll of plant leaves

The effect of the interaction between shade and biological fertilizer was not significant (p>0.05) on the total chlorophyll of upland rice leaves observed at 56 DAA. Table 3 shows the total chlorophyll value for each treatment for each factor. The average total chlorophyll of plant leaves giving shade was 26.53 mg/L, whereas in the treatment without shade it was 20.42 mg/L. The study clearly demonstrates that upland rice plants exhibit greater average total chlorophyll under 29.9% shade compared to the control. However, the two isolates of *Trichoderma* bio-fertilizers showed contrasting results. While the *Trichoderma* Tc-Jjr-02 treatment resulted in a decrease of 12.0% in the average total chlorophyll, the *Trichoderma* Tc-Jro-01 treatment showed an increase of 11.6%.

Total carotenoid leaves of plants

The shade had a significant effect (p <0.05) on the total upland rice leaf carotenoids, Shade had a significant effect (p<0.05) on the total carotenoids of upland rice leaves, meanwhile the effect of biological fertilizer, and the interaction of these two factors was not significant (p>0.05) on the total carotenoids of upland rice leaves observed at 56 DAA. Table 4 shows the average total carotenoids per treatment for each factor.

In the treatment without shade (control), it appears that the average total carotenoid of upland rice leaves was 2.67 (µmol/g), while in the shade treatment was 3.75 (µmol /g). In this case the percentage increase in total carotenoids with shade is 40.3% to the control. In the treatment of *Trichoderma* biological fertilizer, the same pattern as the total chlorophyll of plant leaves was observed. The percentage

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increase in the total chlorophyll in the *Trichoderma* Tc-Jjr-02 treatment was -11.1%, while the treatment of *Trichoderma* Tc-Jro-01 was 5.0%.

Plant height and number of tillers indicate the difference in influence between given shade and without shade. The average height of plants under shade is greater than without shade. In addition to the irradiation time, light intensity influences plant growth [22]. Formation of meristems is strongly influenced by dark conditions or under the auspices [23]. Therefore, plants in the conditions under the shade are higher than in the conditions without shade. Light will affect the work of growth regulator compounds; auxin and cytokines in their role in root growth [24] and stem growth [25]. The level of auxin induction which plays a role in metabolism involving photo-oxidation is influenced by light intensity [26, 27]. On the other hand, young tissue growth is influenced by sucrose levels [28]. The low average daily light intensity in this experiment (1.6100-1.8100 lux) (Table 1) has resulted in a decrease in the rate of photosynthesis which has an impact on reducing the total sugars that are useful for plant growth. It is suspected that etiolation in plants under shade is a response to low levels of sucrose [29]. Although plant growth is greater in shade plants, the number of tillers as a representation of biomass production is smaller than plants without shade (Table 2). *Trichoderma* support for increasing plant growth has been proven in this experiment.

Trichoderma stimulates the activity of indole acetic acid [30] for plants and plays a role in promoting early vegetative growth to crop biomass production [17, 31]. In this acid soil condition, *Trichoderma* enabled plants to produce a number of tillers of 5.75 compared to the controls in the no shade conditions which was an average of 4 at 56 DAAs (Table 2).

Overall, the low growth is thought to be due to the low pH. Too acidic soil will cause fixation of anions such as phosphate and sulfate [32] so that plant growth is generally not optimal. Although the Situpatenggang rice variety is usually cultivated on entisol soil, the average soil pH is <5.0 so that the growth and production of Situpatenggang upland rice is less than optimal, even though it is assisted by the activity of *Trichoderma* which is resistant to acid soil. The Situpatenggang variety requires an optimal pH of 5.5-7.5 so that it can show rapid initial growth [2]. In various regions in Indonesia, entisol soil has physical characteristics with a relatively wide spectrum of pH and texture [33] which fulfill the growing requirements for the Situpatenggang variety. The *Trichoderma* fungi used in this experiment were more tolerant and favored an acidic atmosphere compared to pH 6.5-7.5 [13, 34, 35], assisted plants in overcoming soil acidification [36]; thus, the application of *Trichoderma* as an amendment to the land has increased agronomic productivity of the land [37]. Compost as a "carrier" for Trichoderm isolates, as the active ingredient in the formula used in this experiment is actually an organic material that is the target of

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decomposition by this biological agent fungus, so that the final result of the decomposition process is beneficial for increasing plant growth [38]. It is hoped that this fungal biological agent can be used by farmers to help upland rice which is intercropped between plantation forest stands and people's gardens on acid soils.

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

The interaction between shade and *Trichoderma* bio-fertilizers significantly affected the number of tillers at 21 and 56 days after application of the treatment. Shade and *Trichoderma* biological fertilizer affect the number of tillers but have no effect on plant height 21-56 days after application. Shade affects total carotenoids but does not affect total chlorophyll. Application of *Trichoderma* without fertilizer shows the highest number of tillers, and is able to help upland rice plants overcome acid soil stress in the vegetative phase. Shade of 60% can increase 29.8% of total chlorophyll and 40% of total leaf carotenoid of upland rice plants. The *Trichoderma* biological agent has the potential to be used in rice cultivation in acidic rice fields which often lack water in the dry season, and in upland rice in plantation forests in order to optimize land use and protect plants through the critical period of vegetative growth.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest, or non-financial interest in the subject matter or materials discussed in this manuscript.

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Table 1: Average weekly intensity of sunlight and air temperature above the canopy between 14-56 DAA

Treatment	-th DAA						
	14	21	28	35	42	49	56
The intensity of the light above the canopy under shade of 60% (lux)	165x 100	167x 100	175 x 100	162 x 100	162 x 100	181x 100	162x 100
The intensity of the light above the canopy without shade (lux)	285x 100	278x 100	295 x 100	282 x 100	294 x 100	281x 100	278x 100
The air temperature above the canopy under shade of 60% (^{0}C)	30.4	30.3	30.4	30.1	29.5	30.5	30.2
The air temperature above the canopy without shade (^{0}C)	32.2	31.8	32.3	32.8	31.2	32.6	32.5

Table 2: Effect of combination of shade and *Trichoderma* bio-fertilizers at 28 and 56 DAA on the average number of tillers

Treatments	28 DAA	56 DAA
Without Shade – Without *Trichoderma*	0.50 b	4.00 ab
Without Shade - *Trichoderma* Tc-Jjr-02	0.88 b	5.75 a
Without Shade - *Trichoderma* Tc-Jro-01	1.88 a	5.75 a
Shade 60% - Without *Trichoderma*	0.13 b	2.00 b
Shade 60% - *Trichoderma* Tc-Jjr-02	0.13 b	2.63 b
Shade 60% - *Trichoderma* Tc-Jro-01	0.13 b	3.00 b
HSD 5%	0.84	2.22

The same letters following the numbers in one column indicate that they are not significantly different based on the HSD test at the 5% level

Table 3: The mean of total chlorophyll of upland rice leaves (mg/L) in response to shade and *Trichoderma* biological fertilizer at 56 DAA

Treatment	The mean of total chlorophyll (mg/L)	Percentage increase in total chlorophyll to control
Without shade (control)	20.42	
With shade	26.53	29.9%
Without *Trichoderma* (control)	23.52	
Trichoderma Tc-Jjr-02	20.66	-11.1%
Trichoderma Tc-Jro-01	26.25	11.6%

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Table 4: The mean of total carotenoid of upland rice leaves (μmol / g) in response to shade and *Trichoderma* biological fertilizer at 56 DAA

Treatment	The mean of total carotenoid (μmol/g)	Percentage increase in total carotenoids to control
Without shade (control)	2.67	
With shade	3.75	40.3%
Without *Trichoderma* (control)	3.28	
Trichoderma Tc-Jjr-02	2.91	-11.1%
Trichoderma Tc-Jro-01	3.44	5.0%

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