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Growth and production of chili (*Capsicum annuum* L.) on the application of *Trichoderma* sp. and Azolla liquid organic fertilizer

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Abstract. This study aimed to determine the response of the application *Trichoderma* sp. and Azolla as liquid organic fertilizer on the growth and production of chili (*Capsicum annuum* L.) plants. The study was conducted in Panyili Village, Palakka District, Bone Regency, South Sulawesi Province, from October to December 2017. The research was conducted in the form of an experiment using a split plot design. Two levels of Trichoderma sp. application were set as main plots consisted of control and application of 4 g per plant *Trichoderma asperellum*. Application of Azolla as liquid fertilizer was set as sub plot with five doses, namely control, 15, 30, 45, 60, and 75 ml L^{-1}. The results show that application of *Trichoderma asperellum* 4 g per plant showed the best results on the number of leaves 70 days after planting (DAP) (272.22 leaves). Azolla liquid organic fertilizer (75 ml L^{-1}) resulted in the highest production per plot at harvest period of I-III (1.28 kg/m^2), production per hectare at harvest period of I-III (4.27 tons ha^{-1}). The interaction between *Trichoderma asperellum* 4 g $plant^{-1}$ with a dose of Azolla liquid organic fertilizer of 75 ml L^{-1} showed the lowest attack intensity of aphids (10%).

1. Introduction

Chili (*Capsicum annuum* L.) is one of the horticultural plants that has the potential to be developed in Indonesia. Chili contains nutrients including calories, protein, fat, carbohydrate, calcium, vitamins A, B1 and C [1]. This plant plays an important role in the health field because it functions as an antioxidant and anti-inflammatory which is derived from chili carotenoids. Chili plants are also used for therapy and treatment of arthritis, rheumatism, abdominal pain, skin rashes, and snake bites [2].

Based on the Central Bureau of Statistics [3], the chili still has low in productivity of 8.04 tons ha^{-1}. The gap in productivity of chili products is not only caused by the cultivation of plants that have not been intensive, the influence of climate, the use of seeds, but also the suboptimal use of fertilizers. Chili cultivation is carried out at the farmer level highly dependent and relies on chemical fertilizers continuously. In some cases excessive use of chemical fertilizers will have an impact on the vulnerability of plants to pests and diseases. Excessive use of chemical pesticides also has an impact on increasing pest resistance in chili. One possible solution to this problem is the application of an organic farming system with a balanced fertilizer dose [4].

Research on fertilizer recommendations for chili plants in open fields has been carried out by the Vegetables Research Institute (Balitsa) in Indonesia. Recommendation for fertilizing chilli on dry land is 151 kg N ha^{-1}, 69 kg P_2O_5 ha^{-1}, 120 kg K_2O ha^{-1} while fertilizing chili plants in wetland is 60.3 kg N ha^{-1}, 69 kg P_2O_5 ha^{-1}, and 100 kg K_2O ha^{-1}[5].

To meet fertilizer recommendati[13]s by Balitsa, an alternative is needed by reducing the dose of artificial chemical fertilizatio[13]and the use of liquid organic fertilizer to ensure sufficient nutrient [16]uirements in chili plants. The advantage of using liquid organic fertilizer is that it can overcome nutrient deficiencies and be able to provide nutrients quickly. Liquid organic fertilizer generally does not damage the soil and plants even though it is used as o[13]n as possible [6,7].

One of the ingredients that can be used as a source of liquid organic fertilizer is Azolla. Azolla is a type of floating nail plants, there are lots of water that stagnates, especially in rice fields and ponds [8]. The use of Azolla for lowland rice cultivation can add 20-40 kg N ha^{-1} to the soil and can increase rice yield by 19.23% or 0.5 ton ha^{-1}. If the use of Azolla is given twice, namely before and after planting, an increase in rice yield can reach 38.46% or 1 ton ha^{-1} [9].

Azolla has various nutrients including N (1.96-5.30%), P (0.16-1.59%), K (0.31-5.97%), Si (0.16-3.35%), Ca (0.31-5.97%), Fe (0.04-0.59%), Mg (0.22-0.66%), S (0.22-0.73 %), Na (0.16-1.31%), Cl (0.62-0.90%), Al (0.04-0.59%), Co (0.264 ppm), Zn (26-989) ppm), and Mn (66 - 2944 ppm) [10]. Research on liquid organic fertilizer using Azolla conducte[24]by Suryati et al., [11] testing several concentrations of liquid azolla fertilizer (*Azolla pinnata*) on the growth of oil palm seedlings (*Elaeis guineensis* Jacq.) In the main nursery with a concentration of 125 g L^{-1} showed the best results for increasing seedling height, increasing number of leaves, leaf area, root length, root volume, root canopy ratio and dry weight of the seedlings compared to concentrations of 50, 75, 100 and 150 g L^{-1}.

In addition to the effort to increase soil fertility and support plant growth, control on the attack of pathogenic organisms that cause plant diseases is necessa[9]. One potential way to reduce the use of chemical pesticides is use of *Trichoderma* sp. The fungi *Trichoderma* sp. given to the planting area acts as a biodecomposer, decomposes org[5]ic waste (shed leaves and old twigs) into quality compost and can act as [5]ofungicides, which play a role in controlling pathogenic organisms that cause plant diseases [12]. *Trichoderma* sp. can inhibit the growth of some fungi that cause disease in plants including *Rigidiforus lignosus, Fusarium oxysporum, Rizoctonia solani, Sclerotium rolfsi* [13]. Besides the ability as a biological controller, *Trichoderma* sp. gives a positive influence on plant roots, plant growth and crop production [14]. Other previous study showed that the use of endophytic fungi *Trichoderma* sp. can control the rotten *Phytophthora* (BBP) caused by *Phytophthora palmivora* which is one of the important diseases that play a role in decreasing cocoa production [15]. Application of Trichoderma asperellum at a concentration of 4 g L^{-1} can inhibit the emergence of BBP about 50% in the rainy season. This st[15]y shows the potential use of *Trichoderma asperellum* as a biolog[2]al control agent for *P. palmivora*. Based on this description, a research was conducted to study the growth and production of chili (*Capsicum annum* L.) on the application of *Trichoderma* sp. and Azolla liquid organic fertilizer.

2. Methodology

2.1. A[11]perimental design

This research was conducted in Panyili Village, Palakka District, Bone Regency, South Sulawesi Province from October to December 2017. The trial was set using split plot design (SPD) consisting of two factors namely application [6]f *Trichoderma* sp as the main plot and application of Azolla liquid organic fertilizer as the subplot. The application of *Trichoderma* sp. consisted of two levels, nam[10]y control or without application of *Trichoderma* sp. (t1) and application of *Trichoderma asperellum* at a dose of 4 g $plant^{-1}$ (t2). The application o[4]Azolla liquid organic fertilizer consisted of five levels, namely cont[23]or 0 ml L^{-1} (p0); 15 ml L^{-1} (p1); 30 ml L^{-1} (p2); 45 ml L^{-1} (p3); 60 ml L^{-1} (p4); and 75 ml L^{-1} (p5). Based on the number of treatments tested, 12 treatment combinations were obtained and repeated three times resulted in a total of 36 experimental plots.

The materials used in this study were chili seeds of Batalion variety, *Trichoderma asperellum*, azolla liquid organic fertilizer, fungicide and manure. The tools used in this study were cultivators, hoes, drip hoses, plastic mulch, shovels, scales, buckets, bamboo posts, spray tanks, plastics, cameras, rulers, treatment boards, and writing instruments.

2.2. Preparation of Azolla liquid organic fertilizer

The basic ingredient used as liquid organic fertilizer was Azolla. 3 kg of Azolla leaf were cleaned finely chopped/blended then placed in a 50 L bucket previously filled with 15 L of water and ± 100 ml sugar cane water/brown sugar. The solution was stirred until it homogeneous. 150 ml EM4 were prepared for decomposition microbes and left for ± 30 minutes before added to the solution. A hole was made on the bucket lid (composter) to install the hose where for gas disposal. The solution was stirred slowly and the bucket was closed to avoid nutrients loss due to evaporation. The tip end of the hose connected to the lid was put into a bottle filled with water to remove excess gas from the bucket. The solution was then filtered after storage for 7 days. The solution after filtering is called liquid organic fertilizer and can be used for plants [16].

2.3. Application of treatments

Trichoderma sp. given according to treatment in the planting beds before planting. The Azolla liquid organic fertilizer of 15 ml, 30 ml, 45 ml and 75 ml were dissolved into 1 liter of water in a spray tank prior to application. The Azolla fertilizer was applied on the ground and on the plant. Application of the fertilizer on the ground was carried out by spraying the trial plot. Application of the fertilizer on the plant was carried out by spraying both leaves and stems of the plants. Fertilization is applied in the morning, which is around 06.00 - 08.00 am or in the afternoon at 16.00 – 17.30 pm. These application were started at 10 days after planting (DAP) to 60 DAP and conducted every 10 days (6 times).

2.4. Data analysis

Data were analyzed using analysis of variance for split plot design. The treatment with significant effect was tested further for difference in means using Least Significance Difference (LSD) test at the level of $\alpha = 0.05$.

3. Results and discussion

3.1. Plant height

The results of the ANOVA showed that the treatment of Azolla liquid organic fertilizer significantly affected the plant height parameter of the chilli plants at 70 DAP. No significant effect of both the application of *Trichoderma* sp. and the interaction of the two factors. The average plant height of the chili plant at 70 DAP on different dose of Trichoderma and Azolla liquid organic fertilizers are shown in table 1. Table 1 shows that the treatment of liquid organic fertilizer dosage 30 ml L^{-1} resulted in the tallest plant at 70 DAP (70.39 cm), which was significantly different from the control (63.22 cm) but not significantly different from the other liquid organic fertilizer doses.

Table 1. Average of plant height (cm) 70 DAP of chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and azolla liquid fertilizer

Azolla liquid fertilizer dose	*Trichoderma asperellum* dose		Mean	$LSD_{0.05}$
	0 g $plant^{-1}$	4 g $plant^{-1}$		
0 ml L^{-1}	62.00	64.44	63.22b	4.12
15 ml L^{-1}	69.67	68.56	69.11a	
30 ml L^{-1}	70.89	69.89	70.39a	
45 ml L^{-1}	69.67	70.00	69.83a	
60 ml L^{-1}	71.44	67.78	69.61a	
75 ml L^{-1}	70.67	68.22	69.44a	

Numbers followed by different letters (a, b) mean significantly different in the LSD test $\alpha = 0.05$. DAP = days after planting.

Based on the results of the study, the treatment of Azolla liquid organic fertilizer of 75 ml L^{-1} gave a better growth response compared to the control. The differences occurred may be due to nutrient uptake needed by large chili plants to growth was less available in the control treatment. Azolla liquid fertilizer that has been analyzed contains a total N of 1.645%, a total P of 0.071%, a total K of 2.366% and a Mg of 0.089% [17].

3.2. Number of leave

Analysis of variance shows that number of leaves of the chilli plant were affected significantly by the dose of *Trichoderma asperellum* and Azolla liquid fertilizer. However, no significant interaction of the two treatments found. Table 2 shows that the dose of liquid organic fertilizer 75 ml L^{-1} has the highest average number of leaves at 70 DAP (291 leaves), which was significantly different from the dose of liquid organic fertilizer 0 ml L^{-1} and the dose of liquid organic fertilizer 60 ml L^{-1} but not significantly different from other doses of liquid organic fertilizer. The dose of liquid organic fertilizer 0 ml L^{-1} has the lowest average number of leaves (63.22 leaves). The treatment of *Trichoderma asperellum* 4 g plant $^{-1}$ had the highest average number of leaves (272.22 leaves) which was significantly different from the treatment without *Trichoderma* sp. (225.44 leaves).

Table 2. Average number of leaves (leaves) 70 DAP of chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and Azolla liquid fertilizer

Azolla liquid fertilizer dose	*Trichoderma asperellum* dose		Mean	$LSD_{0.05}$ Azolla fertilizer
	0 g plant^{-1}	4 g plant^{-1}		
0 ml L^{-1}	194.67	216.67	245.67^{c}	48.43
15 ml L^{-1}	201.67	300.00	250.83^{abc}	
30 ml L^{-1}	216.33	306.67	261.50^{ab}	
45 ml L^{-1}	219.00	271.67	245.33^{abc}	
60 ml L^{-1}	250.67	226.67	238.67^{bc}	
75 ml L^{-1}	270.33	311.67	291.00^{a}	
	224,44^{y}	272,22^{x}		
$LSD_{0.05}$ *Trichoderma* sp	45.41			

Numbers followed by different letters in a same column (a, b, c) and row (x, y) mean significantly different in the LSD test $\alpha = 0.05$. DAP = days after planting.

The differences occurred due to the ability of roots to absorb nutrients for the formation of tissue in plants. *Trichoderma* sp. can help the root to absorb certain nutrients especially those needed for tissue formation in the leaves. Antagonistic fungi, especially *Trichoderma* sp., can increase plant germination, number of leaves, leaf area, and dry weight of plants [18,19]. *Trichoderma* sp. helps the parent plant to absorb certain nutrients. According to Sutanto [20] *Trichoderma* sp. is able to break down organic matter derived from plant remnants and be able to increase nutrients N. However, activity of *Trichoderma* sp. was influenced by the environment including pH and temperature [21].

3.3. Number of productive branches, fruit length, fruit weight, and weight of fruit per plant.

Analysis of variance shows that the treatment *Trichoderma asperellum* and Azolla liquid fertilizer doses did not affect the parameters of number of productive branches, fruit length, fruit weight, and weight of fruit per plant of the chili plants. The average values of these parameters are shown in Figure 1. Figure 1 shows that the treatment of *Trichoderma asperellum* 4 g plant^{-1} and a dose of liquid organic fertilizer of 45 ml L^{-1} produced the highest number of productive branches (46.67 stems) while the control treatment of the fungi and the fertilizer resulted in the lowest number of productive

branches (34 stems). The treatment of *Trichoderma asperellum* 4 g plant^{-1} with a dose of Azolla liquid organic fertilizer of 60 ml L^{-1} produced the highest fruit length of 14.51 cm while in the treatment without *Trichoderma* sp. with a dose of liquid organic fertilizer 15 ml L^{-1} (t1p1) produced the lowest length of fruit of 13.44 cm. Treatment of *Trichoderma asperellum* 4 g plant^{-1} with a dose of liquid organic fertilizer of 60 ml L^{-1} (t1p4) produced the highest fruit weight of 13.40 g while in the treatment without *Trichoderma* sp. with a dose of liquid organic fertilizer of 45 ml L^{-1} (t1p3) produced the lowest fruit weight of 12.23 g. The treatment of *Trichoderma asperellum* 4 g plant^{-1} with a dose of liquid organic fertilizer of 60 ml L^{-1} (t1p4) produced the highest fruit weight per plant which was 242.1 g while in the treatment without *Trichoderma* sp. and the dose of liquid organic fertilizer 60 ml L^{-1} (t1p4) produced the lowest fruit weight per plant that is 162.9 g.

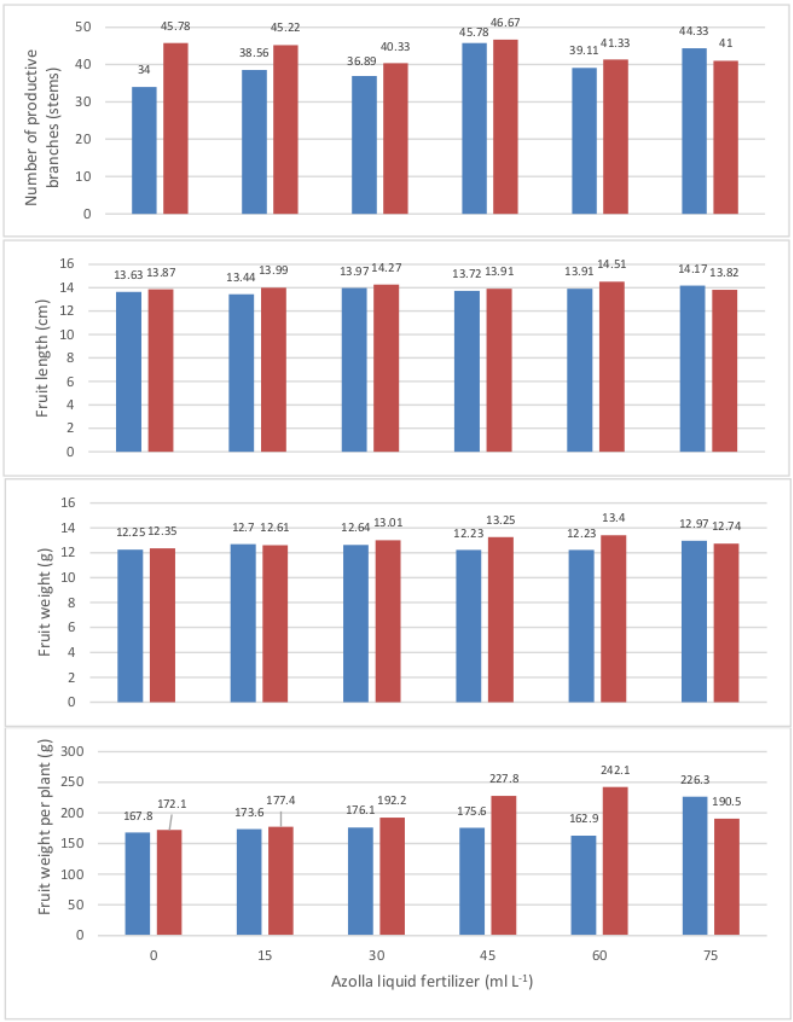

Figure 1. Average number of productive branches (stems), fruit length (cm), fruit weight (g), and weight of fruit per plant (g) of chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and Azolla liquid organic fertilizer.

Trichoderma sp. effect in the recent study was shown not significant mostly on the production parameter. This might due to sub optimal environmental conditions that can influence the effectiveness of *Trichoderma* sp. to function. This is consistent with the opinion of Simarmata et al. [22] that the effectiveness of the fungus *Trichoderma* sp. related to various abiotic soil environmental factors (nutrient concentration, pH, water level, temperature, soil treatment, and fertilizer/pesticide use) and biotic factors (microbial interactions, fungi species, host plants, and competition between *Trichoderma* sp. fungi).

Another possibility is due to the variability of the response of the host plants to the colonization of *Trichoderma* sp. Each combination of the fungi on the host plants have different functions and responses to plants in terms of total P uptake, growth and/or reproduction of plants and this difference also significantly affected plant interactions in an ecosystem [23].

Production per plot.

The results of the variance analysis show that the treatment of Azolla liquid organic fertilizer significantly affected the production per plot of chilli at harvest I-III. No significant effect of the treatment of *Trichoderma* sp. and the interaction between the two factors on the parameter. Average production per plot at harvest I-III as response to the application of *Trichoderma* sp. and Azolla liquid organic fertilizer treatments are shown in table 3. Table 3 shows that the dose of Azolla liquid organic fertilizer of 75 ml L^{-1} resulted in the highest production per plot at harvest I-III which was 1.28 (kg/m^2) and significantly different from the control (0 ml L^{-1} Azolla liquid organic fertilizer) which resulted in the lowest production per plot at harvest I-III (0.88 kg/m^2).

Table 3. Average of production per plot (kg m^2) of chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and Azolla liquid fertilizer.

Azolla liquid fertilizer dose	*Trichoderma asperellum* dose		Mean	$LSD_{0.05}$
	0 g $plant^{-1}$	4 g $plant^{-1}$		
0 ml L^{-1}	0.79	0.97	0.88^{c}	0.25
15 ml L^{-1}	1.04	1.02	1.03^{bc}	
30 ml L^{-1}	1.28	1.09	1.19^{ab}	
45 ml L^{-1}	1.20	1.20	1.20^{ab}	
60 ml L^{-1}	1.17	1.14	1.15^{ab}	
75 ml L^{-1}	1.51	1.05	1.28^{a}	

Numbers followed by different letters (a, b) mean significantly different in the LSD test $\alpha = 0.05$. DAP = days after planting.

Chili plants can grow and produce optimally if the nutrients needed for growth and production are sufficient. Application of macro nutrients (N, P, K, and Mg) to chili plants is very necessary because macro nutrients are nutrients needed in large quantities that play an important role as feeds for plants. The provision of these nutrients in plants is very influential on the photosynthesis process which will affect the growth and production of hybrid chili plants [24]. According to Hardjowigeno [25], the N element contained in the fertilizer functions to stimulate vegetative growth of plants so that plants can synthesize amino acids and proteins and chlorophyll. P element in the fertilizer also serves to stimulate the growth of chilli. While the Mg element can function in the physiological processes of plants results in increased production. As well as the element K which plays a role in the process of photosynthesis so that chilli plants can initiate flowers that in turn affect the dry weight of the plants [24].

3.5. Production per hectare.

The analysis of variance on the parameter of production per hectare at harvest I-III of chili showed that the parameter was significantly affected by the application of Azolla liquid organic fertilizer. While the treatment of *Trichoderma* sp. and the interaction of two factors had no significant effect. Average production per hectare at harvest I-III on different dose of *Trichoderma* sp. and Azolla liquid organic fertilizer treatment are shown in table 4. Based on LSD test at the level of α=0.05, average production per hectare at harvest I-III in table 4 shows that the dose of Azolla liquid organic fertilizer of 75 ml L^{-1} (p5) produced the highest production per hectare (4.27 tons ha^{-1}) which was significantly different from the control treatment (p0) which showed the lowest production per hectare (2.95 tons ha^{-1}).

Table 4. Average of production per hectare (ton $hectare^{-1}$) of chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and Azolla liquid fertilizer.

Azolla liquid fertilizer dose	*Trichoderma asperellum* dose		Mean	$LSD_{0.05}$
	0 g $plant^{-1}$	4 g $plant^{-1}$		
0 ml L^{-1}	2.65	3.24	2.95^{c}	0.82
15 ml L^{-1}	3.48	3.40	3.44^{bc}	
30 ml L^{-1}	4.26	3.65	3.95^{ab}	
45 ml L^{-1}	4.00	4.00	4.00^{ab}	
60 ml L^{-1}	3.89	3.79	3.84^{ab}	
75 ml L^{-1}	5.04	3.50	4.27^{a}	

Numbers followed by different letters (a, b) mean significantly different in the LSD test α = 0.05. DAP = days after planting.

Production per plot, hence production per hectare, is thought to be influenced by vegetative growth of the plant including plant height and the number of leaves of the chili plant. The crop yields are affected by the vegetative growth period experienced by plants, optimal vegetative growth can result in maximum yield. In addition, the amount of fertilizer given is related to the plant's need for nutrients, the content of nutrients present in the soil, and the levels of nutrients contained in the fertilizer. Plant fruit size is determined by vegetative growth before flowering. Thus, for high production, it is necessary to pay attention to its vegetative growth [26].

3.6. Intensity of Aphids attack.

The analysis of variance shows that the treatment of *Trichoderma* sp., Azolla liquid organic fertilizer, and their interactions had significant effect on the intensity of aphids attack on the chili plant. The average intensity of aphids attack on different doses of *Trichoderma* sp. and Azolla liquid organic fertilizer treatment are shown in table 5. Table 5 shows that the lowest intensity of aphids attack was observed on the application of *Trichoderma asperellum* 4 g $plant^{-1}$ treatment with a dose of 45 and 75 ml $L^{-1}$of Azolla liquid organic fertilizer (10%) compared to *Trichoderma* sp. and Azolla liquid organic fertilizer control treatments produced the highest intensity of aphids attack (36.67%).

Table 5. Average intensity of aphids attack on chilli (*Capsicum annuum* L.) on different dose of *Trichoderma asperellum* and Azolla liquid fertilizer.

Azolla liquid fertilizer dose	*Trichoderma asperellum* dose		$LSD_{0.05}$ Azolla fertilizer
	0 g $plant^{-1}$	4 g $plant^{-1}$	
0 ml L^{-1}	$36.67(6.05)^{a}$x	$20.00(4.53)^{a}$y	0.80
15 ml L^{-1}	$30.00(5.47)^{ab}$x	$23.00(4.86)^{a}$y	
30 ml L^{-1}	$26.67(5.19)^{bc}$x	$16.67(4.10)^{a}$x	
45 ml L^{-1}	$30.00(5.47)^{ab}$x	$10.00(3.24)^{b}$y	

60 ml L^{-1}	20.00(4.53)^{c}x	20.00(4.53)^{a}x
75 ml L^{-1}	20.00(4.53)^{c}x	10.00(3.24)^{b}y
$LSD_{0.05\ Trichoderma\ sp}$	0.12	

Numbers followed by different letters in a same column (a, b, c) and row (x, y) mean significantly different in the LSD test $\alpha = 0.05$.

Interaction between *Trichoderma* sp. with Azolla liquid organic fertilizer can reduce the intensity of aphids attack. Application of *Trichoderma* sp. with a dose of 4 g tan^{-1} along with a dose of Azolla liquid organic fertilizer of 75 ml L^{-1} showed relatively low pest attack at 10%. This is likely due to the role of *Trichoderma* sp. as a fungicide that can suppress the growth and development of disease in plants and the growth of plant pathogens that are in the soil or around plant roots. Coupled with the combination of treatment with Azolla liquid organic fertilizer. The effect of solid organic fertilizer in addition to being able to increase the population of beneficial soil organisms that play a role in maintaining soil health, can also suppress various diseases and improve plant health [27].

The highest intensity of aphids attack is found in the control treatments both for *Trichoderma* sp. and the Azolla liquid fertilizer. Absence of the fungi treatment on the plant allows the pest to attack the plant as *Trichoderma* sp. can function as a fungicide that can suppress the growth and development of disease in plants and the growth of plant pathogens that are in the soil or around plant roots [28].

In addition, there is no advantages taken from Azolla liquid organic fertilizer, so that the nutritional needs that should be needed by plants, especially the element of Potassium (K), becomes unbalanced compared to other treatments. This is consistent with the opinion of Nasaruddin [29] the element of Potassium is useful for increasing plant resistance to pests and diseases. Potassium can be absorbed by plants in the form of K^{+} ion. Potassium is found in many young cells or plant parts that contain lots of protein, while the cell nuclei do not contain potassium. In these cells there are some ions in the cell fluid, and such conditions are the most important part in carrying out turgor pressure caused by osmotic pressure.

4. Conclusions

Based on the results of this study concluded that

a) *Trichoderma asperellum* 4 g $plant^{-1}$ gives the best results on the number of leaves at 70 HST.
b) Azolla liquid organic fertilizer (75 ml L^{-1}) gives the best results on the number of leaves of the chili at 70 DAP, production per plot at harvest I-III, the highest production per hectare at harvest I-III.
c) Interaction between *Trichoderma asperellum* 4 g $plant^{-1}$ with a dose of liquid organic fertilizer 75 ml L^{-1} showed the best results at the lowest aphids attack intensity.

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