Capsaicin Level and Anatomy Response of Curly Red Chili (*Capsicum annuum* L.) to Bio Fertilizer and Sludge Biogas Application

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Abstract. Curly red chili (*Capsicum annuum* L.) is widely cultivated using inorganic fertilizers which causes high production cost. Chili is value because of the level of spiciness. The level of spiciness of chili is determined by the level of capsaicin which is affected by nutrients in the growing media. Bio fertilizer and sludge biogas which is one of the organic fertilizer containing microbes and organic material that are able to support nutrien of the medium. This study aimed to measure capsaicin level and analyze the anatomy response of fruits in curly red chili which given by bio fertilizer and sludge biogas in various dosage. The study design used RCBD, the data analyzed by ANOVA at 95% confidence level. The highest capsaicin level were obtain on curly red chili which were given sludge biogas 36 mL + bio fertilizer 10 L /ha. The combination treatment of sludge biogas and bio fertilizer give the highest anatomical response ie thickness of leaves, thickness of parenchyme palisade, diameter of stele, siameter of metaxylem and thickness of pericarp.

Keywords: *Anatomy response, Bio fertilizer, Capsaicin, Chili, Sludge Biogas.*

1 Introduction

Curly red chili (*Capsicum annuum* L.) is an herb that is very popular and has high demand because of its attractive taste, smell and spiciness. The quality and productivity of chilli is strongly influenced by climatic conditions and planting environment. (Kumbar et al., 2017). Chili cultivation is influenced by environmental factors including the intensity of sunlight, rainfall, temperature and humidity, wind, altitude, and soil conditions. The ideal temperature for chili cultivation is 24-28˚C. Chili can adapt well at temperatures of 24-27°C, with moisture that is not too high. Growth will be hampered if the daily temperature in the cultivation area is too cold (Hapsari, 2011). Chili plants require rainfall of 800-2000 mm / year with 80% soil moisture (Tjahjadi, 1991). The height of the place is below 1,400 masl. In the highlands, chili plants can grow but are not optimal. Chili plants are very suitable to be planted on flat land, chili plants can also grow and adapt well to various types of soil, ranging from sandy soil to clay (Harpenas and Dermawan, 2010). Chili plants can grow in soil conditions containing elements essential nutrient elements such as elements N and K and not in a waterlogged condition (Tjahjadi, 1991),

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The use of chemical fertilizers can change biological ecosystems and disrupt the activity of soil microorganisms. Bio-fertilizers are needed to prevent negative impacts from the use of chemical fertilizers. Bio fertilizer able to provide nutrients for plants and increase the level of sustainability of the agronomic system in the long run (Moradi et al., 2011). Bio fertilizer is an organic fertilizer that contains non symbiotic microorganisms that are able to fix N (nitrogen) and provide P (phosphorus) and function as decomposers so they can improve soil quality (Deshmukh et al., 2007), mobilize other main nutrients from unavailable form to a form that can be absorbed and improve the root system. Biological fertilizers can save up to 25-30% of fertilizer application and increase crop production and soil fertility on sustainable agriculture based on ecological principles (Elsen, 2000). Bio fertilizer is an environmentally friendly natural fertilizer with low production costs, increasing growth and yields, improving fruit quality and increasing the efficiency of fertilizer use (Patel et al., 2011).

The productivity of rice in Ngawen rainfed land in Gunung Kidul showed an increase after being given bio-fertilizer treatment. The study also received a bio fertilizer dose of 10-15 liters / ha as the optimum dose for rainfed land (Siswanti et al.,2011). Three rice varieties namely Situbagendit, Ciherang and Inpari 20 had optimal growth and an increase in soil nutrient content after the application of bio-fertilizer in paddy fields affected by Merapi eruption in 2010 (Siswanti & Rahmawati, 2013). Combination of bio-fertilizer and vegetative decomposers have an effect on increasing leaf chlorophyll levels in two rice varieties, Menthik Wangi and Segreng. This combination also provides a good response to the increase in Nitrate Reductase Activity (NRA) on the leaves of the Segreng rice plant flag (Siswanti & Agustin, 2014).

Bio fertilizer was applied together with sludge. Sludge is a by-product of biogas in the form of mud, which contains a lot of nutrients which can be used as fertilizers. Nutrients in sludge can increase soil fertility by improving the physical, chemical and biological properties of the soil. Sludge has undergone anaerobic fermentation so that it can be directly used to fertilize plants, so the use of bio fertilizers along with sludge can optimize the increase in crop productivity (Wahyuni, 2011). Based on the analysis of wet weight, the content in sludge are organic C-48%, N-total 2.9%, C / N 15.8%, P_2O_5 0.2%, K_2O 0.3% (House of Biogas Team, 2013). Sludge has several benefits including increasing the formation of leaf chlorophyll, increasing plant vigor so that plants become sturdy and increasing plant resistance to drought (Rizqiani et al., 2007). Sludge has characteristics that are free of pathogens and capable of killing disease-causing organisms in plants, can reduce the growth of weeds, as a good soil moisturizer because it can add topsoil and increase the water content in the soil (Handaka, 2012).

Improving the quality of production can be observed through plant micromorphological observations to determine the response at the cellular level anatomically (Weryszko-Chmielewska and Michałojć, 2011). Micromorphological observations can be observed in fruit, roots as absorption organs of water and nutrients, and leaves as a place for photosynthesis. The use of bio fertilizer in large red chili plants and curly red chili influences anatomically the thickness of the pericarp, septal thickness, and the number of peripheral septal cells in chili fruit organs, whereas the effect of using bio fertilizer-sludge on chili plants is unknown (Aisyah, 2009). The purpose of this study was to analyze the capsaicin content and anatomical response of curly red chili plants to the application of sludge biogas and bio-fertilizer.

2 Material and Methods

Planting curly red chili is done in April - September 2017 in Wukirsari Village, Cangkringan, Sleman, Yogyakarta. Capsaicin levels were measured at the Organic Chemistry Laboratory of the Faculty of MIPA UGM. While the making and observation of anatomical preparations was carried out in March - April 2019 at the Laboratory of Plant Development Structures, Faculty

of Biology, Gadjah Mada University. The materials needed for preparation of planting are biogas sludge 144 liters, 40 liters bio fertilizer, NPK fertilizer, and curly red chili plant seeds.

Table 1. Research Treatment

No	Perlakuan
1	Control (use NPK fertilizer)
2	Sludge 12 mL
3	Sludge 24 mL
4	Sludge 36 mL
5	Bio fertilizer 10 L/ha
6	Sludge 12 ml + Bio fertilizer 10 L/ha
7	Sludge 24 mL + Bio fertilizer 10 L/ha
8	Sludge 36 mL+Bio fertilizer 10L/ha

Measurement of capsaicin content was carried out by alkaloid extraction method (Nugroho et al., 2002). Curly red chili fruit is taken 2 grams. Each part of the fruit is crushed with mortar until smooth, then added with methanol and 0.01 M HCl (4 mL: 6 mL), put in a test tube. It is cortexed for 2 minutes and sonicated for 30 minutes using an ultrasonicator. The solution was centrifuged for 10 minutes at 4000 rpm. The supernatant is taken, put in a 125 ml separating funnel, plus 10 ml of chlorophome, then shaken up until the two liquids are mixed. Two layers will form. The top layer (water phase) was taken, poured in a porcelain dish, pH was made 11 by adding ± 11 drops of NaOH 1 M. The measured solution was poured in a separating funnel for extraction by adding chlorophome: methanol (9: 1 v / v) Extraction is done 3 times. The water phase (top layer) is removed, the chlorophom phase is accommodated in a porcelain dish, then evaporated. The residue was dissolved with 500 µL / 0.5 mL methanol. Then put in a flask bottle. Extracts are injected into gas chromatography. Gas chromatography analysis using methanol solvent, HP-5 column 5% Phenyl Methyl Sitoxon 30 meters. The initial temperature is 100ºC, the initial time is 2 minutes, the temperature rise is 10˚C per minute, the final temperature is 280˚C. F10 detector type, 380ºC detector temperature, injector temperature 280ºC. The carrier gas is Hl (Hydogen Iodide), total flow is 10, split (kpa) 60, and the amount of injection is 1 µM. The results of GC injection in the form of a curve were observed to determine the presence of capsaicin compounds. The retention time of the sample is matched with standard retention time, the value that is appropriate or approaching is assumed to be a capsaicin compound. Relative capsaicin levels in curly red chili are determined by converting the highest peak area to 100%. The area of the peak below follows the percentage.

The samples used in this study include leaves, roots, and fruit of *Capsicum annuum* L. as a result of application of bio-sludge in various treatments Materials for making preparations namely FAA solution (Formalin, glacial acetic acid and 70% alcohol) for fixation, alcohol 70 %, 80% alcohol, 90% alcohol, 100% alcohol for dehydration and coloring, xylol solution, alcohol: xylol with a ratio of 1: 3; 1: 1; 3: 1, mixture of xylol: paraffin with a ratio of 1: 9 for alcoholization, and pure paraffin for infiltration. For coloring, safranin 1% was used in 70% alcohol, glycerin, balsam canada for preparatory closure.

Sampel were observed using a microscope that had been linked to optilab version 2.2. Image capture is made with optilab preparations. Then the measurements were made using the Image Raster type 3.0 program on various parameters for each preparation. Data from parameter measurement were analyzed descriptively and quantitatively using Analysis of Variance ANOVA, followed by DMRT test with a confidence level of 95% ($\alpha = 0.05$). Quantitative data analysis was performed using SPSS software.

3 Results and Discussion

The environmental conditions of agricultural land at week 8th, when samples of leaves, fruit and roots were taken were 493 lux in the average light intensity, 29ºC soil temperature at 10:00 am with pH 7 and soil moisture in dry conditions. This condition is not optimal for the growth of curly red chili plants, but the morphological conditions and growth of chili plants are still quite good.

The measurement of capsaicin levels was carried out at the maximum vegetative phase of the curly red chili plant which was at the 8th week.

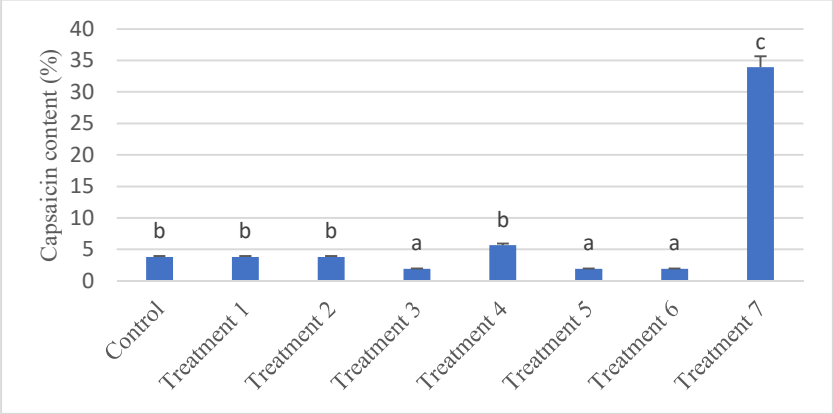

Fig 1. Capsaicin levels of curly red chili fruit (*Capsicum annum*. L.) as a result of bio fertilizer and biogas sludge at 8 th week after planting.

Based on Figure 1, capsaicin levels of curly red chili fruit showed mixed results at each given dose of fertilizer. It can be seen that the most capsaicin levels are in plants with the application of 36 mL sludge biogas and 10 L bio-fertilizer / ha while the capsaicin levels are the least in plants with 36 mL sludge biogas application, 12 mL sludge biogas and 10 L / ha bio-fertilizer, and 24 mL sludge biogas and 10 L bio-fertilizer. When compared with controls, plants with application of bio-fertilizer 10 L / ha and application of 36 mL sludge biogas and 10 L / ha bio-fertilizer were able to increase capsaicin levels of curly red chili plants while the application of bio-fertilizer and sludge biogas with other doses tended to reduce chlorophyll levels. It is known that the most optimal level of capsaicin is found in 36 mL sludge biogas and 10 L / ha bio fertilizer which is the highest dose. This indicates that an increase in the dosage of application of bio-fertilizer and biogas sludge in susceptible plant tolerance can increase capsaicin levels in curly red chili fruit. Based on the analysis of wet weight, the content in biogas waste liquid fertilizer is organic C-48%, N-total 2.9%, C / N 15.8%, P_2O_5 0.2%, K_2O 0.3% (House Biogas Team, 2013). The content of sludge biogas which is rich in carbon and nitrogen makes the vegetative and generative growth of curly red chili plants balanced and faster, so that they can form more secondary metabolites compared to chili control plants. Increasing the dose of nitrogen fertilizer will increase the total content of alkaloids (Lingga, 2001).

The measurement of the thickness of curly red chili leaves is shown in Figure 2.

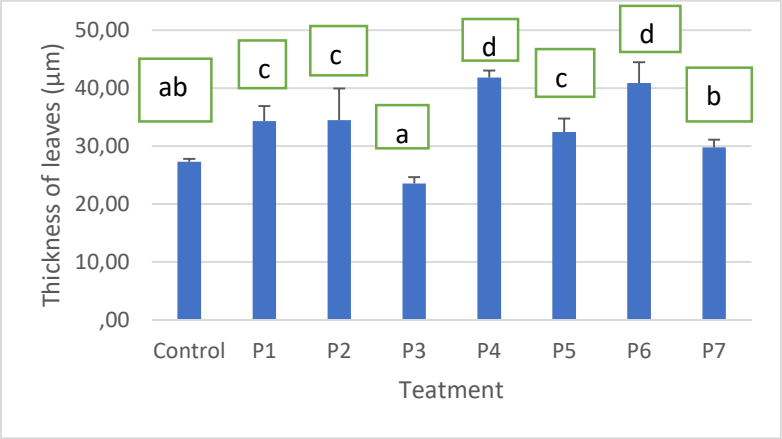

Fig 2. Thickness of leaves of *Capsicum annuum* L.

Based on Figure 2, the highest leaf thickness was in treatment 4, which was giving 24 mL and 6 sludge with 12 mL + bio fertilizer 10 sl / ha sludge treatment.

The thickness of palisade parenchyme tissue in leaves is shown in Figure 3.

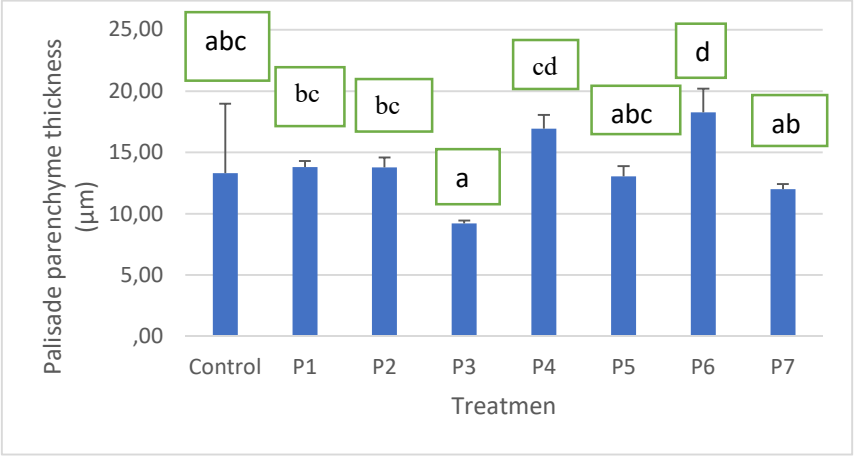

Fig 3. Palisade parenchyme thickness on *Capsicum annuum* L.

Based on Figure 3, plants with treatment 6, which given 24 mL + bio fertilizer 10 L / ha sludge had the highest palisade parenchyme tissue thickness, which was not significantly different from treatment 4, which given bio fertilizer 10 L / ha.

The diameter of stele in root is shown in Figure 4

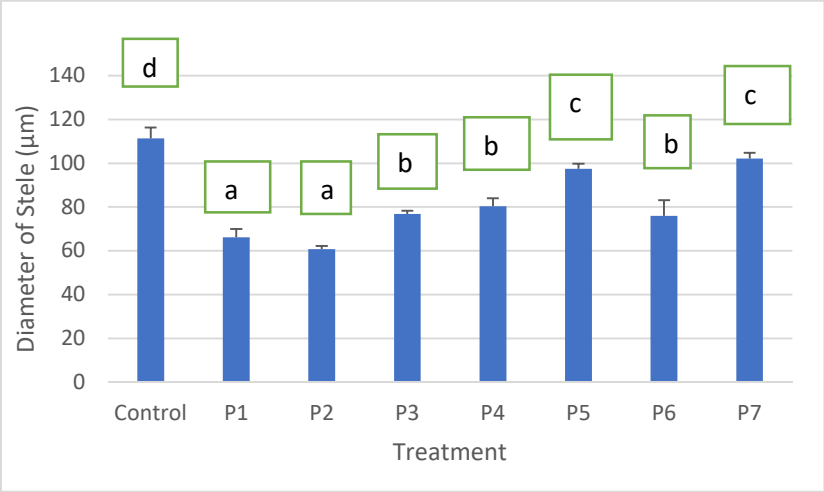

Fig 4. Diameter of stele on *Capsicum annuum* L.

Based on Figure 4, control had the highest diameter of stele, which was significantly different from other treatments.

The diameter of metaxilem in root is shown in Figure 5.

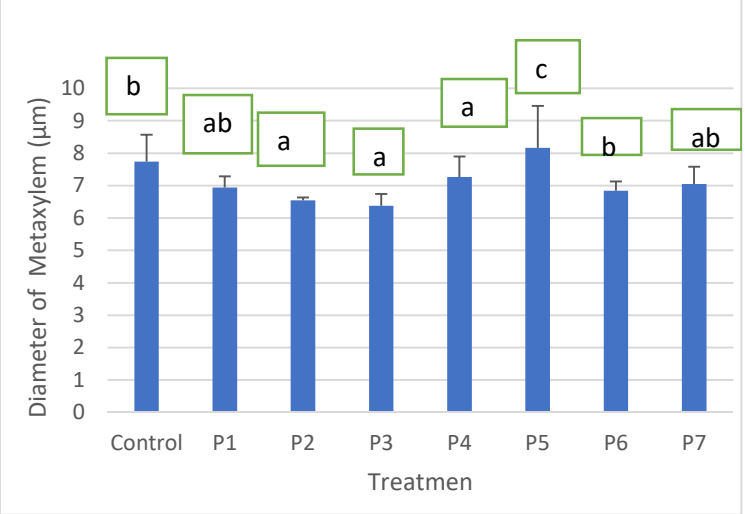

Fig 4. Diameter of metaxylem on *Capsicum annuum* L.

Based on Figure 5, treatmen 5, which given 12 mL sludge biogas and 10 L bio fertilizer had the highest diameter of metaxylem, which was significantly different from other treatments.

The thickness of pericarp in root is shown in Figure 5.

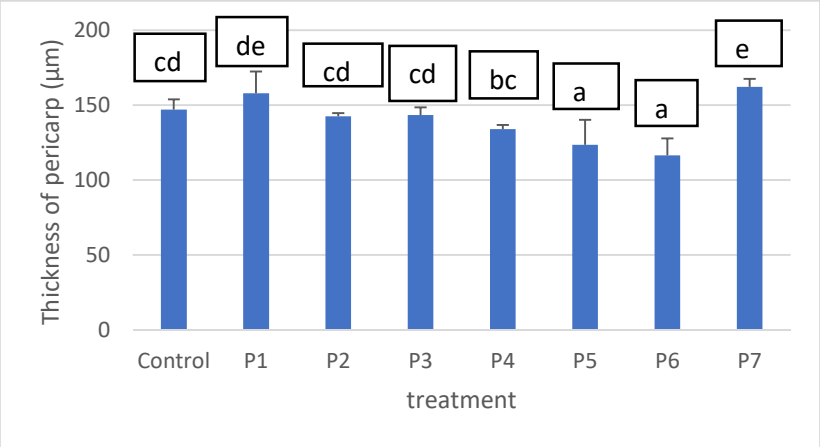

Fig 6. Thickness of pericarp on *Capsicum annuum* L.

Based on Figure 6, treatment 7, which given 36 mL sludge biogas and 10 L bio fertilizer had the highest thickness ofpericarp, which was significantly different from other treatments.

4 Conclusion

Based on the results of the research on capsaicin level and anatomycal response of the curly chili plant to bio fertilizer and biogas sludge application, some conclusions can be drawn that the application of 36 mL sludge biogas and 10 L / ha bio-fertilizer on curly red chili plants has the highest capsaicin levels. The combination treatment of sludge biogas and bio fertilizer give the higest anatomycal response of curly red chili ie thickness of leaves, thickness of parenchyme palisade, diameter of stele, siameter of metaxylem and thickness of pericarp.

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