NUSANTARA BIOSCIENCE
Vol. 1, No. 1, pp. 9-16
March 2009

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n010102

Biomass, chlorophyll and nitrogen content of leaves of two chili pepper varieties (*Capsicum annum*) in different fertilization treatments

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Manuscript received: 28 October 2008. Revision accepted: 27 January 2009.

Abstract. *Suharja, Sutarno. 2009. Biomass, chlorophyll and nitrogen content of leaves of two chili pepper varieties (*Capsicum annum*) in different fertilization treatments. Nusantara Bioscience 1: 9-16.* This study aims to determine the influence of various fertilization treatments on biomass, chlorophyll, and nitrogen content of leaves from two varieties of chili, *Sakti* (large chili) and *Fantastic* (curly chili). The study was conducted in the village of Gatak, Karangnongko sub-district, Klaten District, Central Java, from September 2006 to March 2007. The study used a complete block design with two factorials, chili varieties, and fertilizer treatment. Fertilization treatments includes no fertilizer (control) (P1); manure 2 kg/plant (P2), manure (1 kg/plant) + chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) + NPK (P3); and manure (1 kg/plant) + chemical fertilizer (SP-36: KCl = 1:1) + liquid organic fertilizer (P4). According to Harborne (1987), chlorophyll content was measured, whereas leaf nitrogen concentration was measured with the Kjeldahl method. Data were analyzed using ANOVA followed by DMRT. The results showed that the Fantastic chili fertilizer treatment affected the biomass and chlorophyll-a but did not affect chlorophyll *b*, total chlorophyll, and leaf nitrogen. On the curly chili, fertilizer treatment affected fresh plant weight, chlorophyll *a*, and total chlorophyll but did not affect dry weight, fresh fruit weight, chlorophyll *b*, and leaf nitrogen. It is, therefore, recommended to use the formulation of manure + chemical fertilizer (SP-36: KCl = 1: 1) + liquid organic fertilizer in the cultivation of chili.

Keywords: biomass, chlorophyll, leaf nitrogen, chili, *Capsicum annum*, fertilizing.

Abstrak. *Suharja, Sutarno. 2009. Biomassa, kandungan klorofil dan nitrogen daun dua varietas cabai (*Capsicum annum*) pada berbagai perlakuan pemupukan. Nusantara Bioscience 1: 9-16.* Penelitian ini bertujuan untuk mengetahui pengaruh berbagai perlakuan pemupukan terhadap biomassa, kandungan klorofil dan nitrogen daun dari dua varietas cabai, *Sakti* (cabai besar) dan *Fantastic* (cabai keriting). Penelitian dilakukan di Desa Gatak, Kecamatan Karangnongko, Kabupaten Klaten, Jawa Tengah pada September 2006 sampai Maret 2007. Penelitian menggunakan rancangan blok lengkap dengan dua faktorial yaitu varietas cabai dan perlakuan pemupukan. Perlakuan pemupukan meliputi tanpa pupuk (kontrol) (P1); pupuk kandang 2 kg/tanaman (P2), pupuk kandang (1 kg/tanaman) + pupuk kimia (ZA, SP-36, KCl = 2: 1: 1) + NPK (P3); dan pupuk kandang (1 kg/tanaman) + pupuk kimia (SP-36: KCl = 1:1) + pupuk organik cair (P4). Kadar klorofil diukur merujuk Harborne (1987), sedangkan kadar nitrogen daun diukur dengan metode Kjeldahl. Data dianalisis menggunakan Analisis Varians dilanjutkan DMRT. Hasil penelitian menunjukkan pada cabai *Fantastic*, perlakuan berbagai macam pemupukan berpengaruh terhadap biomassa dan klorofil a, namun tidak berpengaruh terhadap kandungan klorofil b, total klorofil dan nitrogen daun. Pada cabai *Sakti* perlakuan pemupukan berpengaruh terhadap bobot segar tanaman, kandungan klorofil a dan total klorofil, namun tidak berpengaruh terhadap bobot kering, bobot buah segar, kandungan klorofil b dan nitrogen daun. Oleh kerena itu direkomendasikan untuk menggunakan formulasi pupuk kandang + pupuk kimia (SP-36: KCl = 1: 1) + pupuk organik cair dalam budidaya cabai.

Kata kunci: biomasa, klorofil, nitrogen daun, cabai, *Capsicum annum*, pemupukan.

INTRODUCTION

The demand for chili pepper (Capsicum annuum L) always increases with additional food factories, family needs, and various instant noodles, sauce, and chilly industries. Results of the 2007 chili crop were 6.30 tons/ha, which was lower than in 2006 6.51 tons/ha. Meanwhile, the import of pepper in 2006 was 11,885,501 tons, and the export of pepper in 2006 was 8,004,450 tons. This condition implies that the production of large chili per hectare needs to be developed. Therefore, it is necessary to find cultivation technology that can increase the growth and yield of chili (www.hortikultura.deptan.go.id 2008).

According to Nyakpa et al. (1988), the success of a farming business is largely determined by the growth and yield of cultivated plants. If the growth and yield are satisfactory, the business is said to be successful. Allabi (2005) further states that peppers will give good results if the essential elements' needs are met. To meet the needs of the vital elements, fertilization can be done. The successful use of organic fertilizers in encouraging crop production is not doubted. Providing organic fertilizer to increase growth and yield can yield better than phosphorus.

Meanwhile, Sadewa (2008) states that the type of fertilizer mixture (N: P: K = 8.31 g: 12.21 g: 8.81 g) can improve the plant's height, root length, and chlorophyll content of three varieties of chili peppers at the vegetative phase. According to Chellemi and Lazarovits (2002), applying organic fertilizer 310 and 400 kg N/ha may cause a decrease in the production of chili peppers and tomatoes; even providing 560 kg N/ha may lead to increase soil pH, NH3, and the total number of fungus/mushroom, which can be toxic and can cause the death of the pepper.

Based on the researches of Sadewo (2008), Allabi (2005), and Chellemi and Lazarovits (2002), chili pepper plants require macro and micronutrients for growing and increasing production. These needs of nutrients can be met through the provision of organic fertilizer and inorganic fertilizer. Formulation of the appropriate types of fertilizer can affect the biomass, chlorophyll content, and the leaf's nitrogen. The thought that organic fertilizers can increase agricultural production is not entirely correct—only the appropriate formulations of fertilizer can affect the growth and the yield of chili. Therefore, research needs to be done to find a formulation of organic and inorganic fertilizers and/or the combination of both fertilizers and their influence on the pepper plant.

Big or large chili (*C. annuum*) has many varieties, among others: the long red chili (*C. annuum* var. *longum* (DC.) Sendt), round peppers (*C. annuum* var. *Cerasiforme* (Miller) Irish), sweet chili or bell peppers or paprika (*C. annuum* var. *grossum*) and green peppers (*C. annuum* var. *annuum*) and others. Curly chili is one of the varieties of long red chili (*C. annuum* var. *longum* (DC.) Sendt) (Pracaya 2000; Setiadi 1993).

Biomass is defined as the total of life at any given time and in a certain area. Biomass can be expressed as biomass volume, wet weight biomass, dry weight biomass, and organic biomass (Michael 1994). Prawirohatmodjo *et al.* (2001) further state that biomass covers the entire body of a living creature, even when the body is only a branch or a leaf of a tree as long as still attached to the plants. According to Salisbury and Ross (1995), fresh mass is determined by harvesting the whole plants or parts of the plant and weighing them quickly before too much water evaporates from the material. About 75% of plant biomass is produced several weeks before harvest time so that at that time, the plant needs higher nutrients and absorbs fertilizer more efficiently (Rubatzky and Yamaguchi 1995). The measurement of biomass can also use dry mass. Dry mass measurement needs to be done because of various problems arising from the content of the water, so the productivity of cultivated plants should also be measured by using the dry mass of the plants (Salisbury and Ross 1995). Dry weight biomass is measured to obtain the overall appearance of plant growth (Sitompul and Guritno 1995). Measurement of dry weight accumulation is an analogy to know the distribution pattern of assimilation from the source to the target (Gardner *et al.*, 1991).

Chlorophyll is a magnesium-porphyrin attached to proteins (Nelson and Cox 2004). Chlorophyll is an essential catalyst for photosynthesis found in thylakoid membranes as a green pigment in plant photosynthetic tissues, which is loosely bound to proteins but easily extracted into a solvent such as acetone and ether lipids (Harborne 1987). Taller plants have two chlorophyll types: chlorophyll *a* and chlorophyll *b*. Chlorophyll *a* is a complex compound of magnesium and porphyrin, with a cyclopentanone ring (ring V). The four nitrogen atoms are linked by coordination ties with Mg2 +, forming a firm planar compound. The hydrophobic side chaierpenoid alcohol, or phytol, is connected by the ester bonds propionate group of rings IV. Chlorophyll *b* is the second chlorophyll found in plants (Wirahadikusumah 1985). The structure of chlorophyll *b* is different from chlorophyll *a* because chlorophyll *a* has a methyl catalyst. In contrast, chlorophyll *b* has an aldehyde group attached on the right top of the pyrrole ring (Harborne 1987). Chlorophyll *b* may derive from chlorophyll *a*, methyl groups are oxidized on its second ring and become the aldehyde group or are possible for the porphyrin compounds, which can be converted to chlorophyll *a* and *b* (Bonner and Varner 1965). Porphyrin in chlorophyll *a* is the precursor of chlorophyll, both a and b (Mohr and Schopfer 1995).

In-plant tissues, nitrogen is a constituent component of many essential compounds such as proteins, amino acids, amides, nucleic acids, nucleotides, coenzymes (Loveless 1987), chlorophyll, cytosine, auxin (Lakitan 2007), and the main components of dry material derived from protoplasmic material plants (Salisbury and Ross 1992). Plants absorb nitrogen elements in NO^{3-} and NH^{4+} (Nyakpa et al. 1988).

Fertilizers are all materials provided for the ground to improve the physical, chemical, and biological soil condition (Subagyo 1970). Fertilizer is a material given to soil, both organic and inorganic material, to replace the loss of nutrients from the soil and increase crop production (Sutejo 2002). The provision of different types of fertilizer can affect the growth and yield of plants. The yield and quality of paprika will be different depending on the different types of nitrogen fertilizer which is given to it, both PCU (*polyolefin resin-coated urea*) and SCU (*sulfur coated urea*) (Guertal 2000).

Organic fertilizers have a role in influencing the physical properties, chemical, and soil biological activity. Organic fertilizers can improve soil physical characters through the formation of soil aggregate structure and a stable and closely related to water-binding ability, water infiltration, reduce erosion, increase ion exchange capacity (CEC), and as a regulator of soil temperature, all affect the plant growth (Kononova 1999; Foth 1990). Organic fertilizers contain nutrients needed for plant growth (Rauf 1995; Tandisau and Sariubang 1995). The use of organic extract (organic liquid fertilizer) with a concentration of 2-3 mL/L water can increase the yield of various crops, like peppers, tomatoes, and corn by about 25% (Sima 1999, 2005). Giving the organic extract a relatively short interval (7 days) can directly maintain the supply of nutrients and soil microbe vitamins that play a role in the decomposition process of soil organic matter and keep soil healthy (Diver 2001; Scheuerell 2004).

This study aims to know the influence of various fertilizers on biomass, chlorophyll content, and leaf nitrogen in two varieties of chili pepper (*Capsicum annuum* L.), large chili, and curly chili.

MATERIALS AND METHODS

Time and place

This research was conducted from September 2006 until March 2007 in the rice field in Gathak village, Karangnongko sub-district, Klaten District, Central Java. The chlorophyll content and leaf nitrogen measurement were conducted at the Laboratory of Soil Science, Faculty of Agriculture, Sebelas Maret University, Surakarta.

Material

Seeds of large chili from varieties of *Fantastic* and seed of curly chili varieties of *Sakti* are from the same broodstock. It needs fertilizers such as NPK *Mutiara*, cattle manure, ZA, SP-36, KCl, and liquid organic fertilizer branded *Batari Sri*. According to PT. Batari Sri (2005), liquid organic fertilizer (LOF) *Batari Sri* is a pure organic fertilizer that 97% is made from cattle urine and 3% of natural ingredients processed by fermentation to produce liquid fertilizer that does not contain zinc (Zn), copper (Cu) and lead (Pb).

Study design

This research was a factorial with randomized complete block design (RCBD) with 2 factors: (i) variety (two levels) (ii) fertilization (four levels), with 3 replications and each replication consisted of 20 planted chili. Fertilization treatments included: P1 = no fertilizer as control; P2 = manure 2 kg/plant; P3 = manure (1 kg/plant) + chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) + NPK *Mutiara*; and P4 = manure (1 kg/plant) + chemical fertilizer (SP-36: KCl = 1:1) + liquid organic fertilizer *Bathari Sri*.

Research parameter

The parameters of this study are biomass (fresh plant weight, dry weight of plants, and fruit fresh weight per plant), chlorophyll (chlorophyll *a*, chlorophyll *b*, and total chlorophyll), and leaf nitrogen content. Measurement of research variables for parameters is done by taking three plants per block per treatment indicated and measured at the end of harvest.

Plant wet weight was measured by taking the entire plant and weighed. Plant dry weight was measured by drying the wet plants in an oven for 48 hours (until constant weight is obtained). The watery fruit's weight was obtained in every harvest (from harvest 1 to finish). The total weight of fruit is obtained by summing the wet weight of fruit at each harvest. The weight of fruit per plant was calculated by counting the total weight divided by the total number of plants per plot (20 plants). Plant chlorophyll content was measured with a spectrophotometer according to Harborne (1987). Plant leaf nitrogen content was measured by using the method of Sudarmaji *et al*. (1996).

Data analysis

Data obtained from this study were analyzed by analysis of variance (ANOVA) and followed by DMRT (Duncan's multiple range test) using SPSS 10:05. In the analysis of variance, if F was greater than the F table or a probability (sig) <0.05 hence H0 refused, and H1 accepted. DMRT (Duncan) populations with the same average were grouped into one subset. In one subset, the treatment was not different (Hanafi 2005; Pratista 2002).

RESULTS AND DISCUSSION

Fertilization has long been known as influencing the growth and yield of crops, including chili. Fertilizing is an effort to provide the necessary elements of plant nutrients. The provision of nutrients affects the plant's organic and inorganic compounds level (Rosmarkam and Yuwono 2002). Among the parameters that can be observed as a physiological phenomenon are the fresh weight, dry weight, chlorophyll, and nitrogen content of leaves (Marschner 1986).

Biomass

Biomass in this study includes the weight of wet and dry plants at the end of harvest and the total wet weight of fruit per plant.

Plant fresh weight

Fresh weight is the total weight of plants showing metabolic activity results. Fertilization can affect fresh plant weight as it provides nutrients from the soil—the fresh weight of pepper plants at different fertilizer treatments varied (Table 4). The fresh weight of the control group (P1) is equal to the weight of fresh manure treatment (P2), and not in one group with that treated with manure + chemical fertilizer + NPK (P3), and also that have different with that have treatment of chemical fertilizer + manure + liquid organic fertilizer (P4).

The dry weight of the plant

The dry weight of plants of both varieties of peppers varies. Various fertilization treatments significantly affect the increase in plant dry weight of *Fantastic* chili, but no significant effect on the dry weight of *Sakti* chili (Table 5).

Table 1. Biomass plant two varieties of chili

Variety	Biomass			
	P1	P2	P3	P4
Plant fresh weight (g)				
Fantastic	228.93 a	496.01 ab	602.11 b	665.65 b
Sakti	277.16 c	508.33 cd	607.56 d	656.52 d
Plant dry weight (g)				
Fantastic	68.33b	111.11c	139.67cd	157 d
Sakti	126.59a	155.41a	354.28a	407.72a
Total fruit weight per plant (g)				
Fantastic	805.00 a	878.33 ab	770.83a	990.93 b
Sakti	530.83 c	546.67 c	606.67 cd	705.83 d

Note: Number that are noted same on the same line means that they are not significantly different according to DMRT test at P = 0.05. P1: Control, P2: Manure (2 kg/plant), P3: Manure (1 kg/plant) + Chemical fertilizer (ZA: KCl: Sp-36 = 2: 1: 1) + NPK *Mutiara*, P4: manure + chemical fertilizer (KCl: SP-36 = 1: 1) + liquid organic fertilizer *Bathari Sri*.

The absence of influence of various fertilization treatments on the dry weight of chili is a powerful indication that fertilization has not been quite able to meet the nutrient requirements needed by chili. Fertilizer treatment on different varieties of chili shows different effects. This is in line with Kartasapoetra's (1995) statement and Abdul Rahim and Jumiati's (2007) that the plant will need a variety of nutrients for growth and development; it takes them different times.

Although the fertilizer treatment was not significantly different in the dry weight of *Sakti*, it can be seen that there is a tendency that fertilizer treatment can improve the dry weight of both varieties of chili. This means photosynthesis tends to increase due to the addition of nutrients from the soil due to the fertilization process.

Total fruit weight per plant

The total fruit weight per plant from both varieties of chili is different in various fertilizer treatments. The results showed that the fertilization treatment did not significantly affect fruit weight per plant in *Sakti* (Table 1). This indicates that manure, chemical fertilizer, and NPK (P3) is not always a solution to improve the yields. Only the precise formulation increases the weight of pepper. Meanwhile, fertilizer treatment significantly affected Fantastic total fruit weight per plant (Table 1). The presence of N, P, K fertilizer and organic matter deriving from chemical fertilizer and liquid organic fertilizer make it possible to increase the weight of the chili. The combination of manure, chemical fertilizer, and liquid organic fertilizer (P4) can be used to replace chemical fertilizer formulations that farmers in the Klaten district have used.

Meanwhile, in manure (1 kg/plant) + chemical fertilizer (ZA: KCl: SP-36 = 2: 1: 1) + Shake *Mutiara* NPK gave fruit weight per plant that was lower than the other treatments (Table 1). This can happen because the nitrogen in the treatment has exceeded the optimal point, thus causing partial breakaway of assimilated nitrogen as an amide; it just raises the nitrogen content of plants and reduces the synthesis of carbohydrates (Rosmarkam and Yuwono 2005). Therefore, the fresh weight of formed fruit is relatively lower than other treatments.

The increase in the biomass of *Sakti* chili and the tendency of *Fantastic'* chili biomass to increase shows that the elements provided through fertilization function properly. Results of correlation analysis show that fresh weight is positively associated with plant dry weight, chlorophyll *a*, chlorophyll *b*, and total chlorophyll of the two varieties of the chili. This means that if the fresh weight of plants increases, the dry weight will increase too, and so are the chlorophyll *a*, chlorophyll *b*, and total chlorophyll leaves of both varieties of chili.

Organic fertilizers can provide soil organic matter that is very helpful in restoring the fertility of physics, chemistry, and biology of soil because it is useful to bind soil particles through soil aggregation. Aggregation of soil can produce micro-pore space so that the aeration in the soil becomes better, and it creates optimum conditions for absorption of nutrients for plants (Brady 1990). The influence of organic matter on soil chemical fertility, among others, is cation and anion exchange capacity, the increase of soil microbial activity through decomposition, and mineralization of organic matter (Suntoro 2002). Besides, organic material can also absorb and hold water (Juan et al., 2003), which affects the accumulation of nutrients and products of metabolism stored in the fruit and seeds.

Meanwhile, the chemical fertilizers progressively increase and complete nutrients (nitrogen, phosphorus, potassium, magnesium, sulfur) useful in increasing chili plant biomass. The nitrogen (from ZA and NPK*Mutiara*) is capable of acting as a constituent of many essential compounds such as proteins, amino acids, amides, nucleic acids, nucleotides, coenzymes, and many compounds vital to metabolism, a constituent of chlorophyll, cytosine, and auxin hormones and as main components of plant dry matter. Nitrogen will increase the green color of leaves, stems, and leaf growth (Marschner 1986). Nitrogen is closely related to the synthesis of chlorophyll (Sallisbury and Ross 1992) and the synthesis of proteins and enzymes (Schaffer 1996). Rubisco enzyme acts as a catalyst in the fixation of CO2 that plants need for photosynthesis (Salisbury and Ross 1992; Schaffer 1996). Therefore, increasing the nitrogen content of plants can affect good photosynthesis through chlorophyll content and photosynthetic enzymes, thereby increasing the *photosynthat*es (fresh weight, dry weight, and weight of chili pepper) is formed.

The phosphorus (from SP-36) is an essential component of ATP constituent compounds that act as an energy source in the dark reactions of photosynthesis and the nucleoprotein, the genetic information system (DNA and RNA), cell membranes (phospholipids), and phosphoprotein. The KCl Fertilization increases the availability and the absorbance of potassium, while the function of potassium in the chloroplast is to play a role as a guard to keep a high pH. Kalium plays a vital role in photosynthesis because it directly increases the growth and leaf area index, thus increasing the assimilation of CO_2 and increasing translocation and assimilation of photosynthesis results (Suntoro 2002).

Plants need sulfur (from SP-36) to form the amino acids cysteine, cysteine, and methionine. Besides, sulfur is also part of biotin, thiamine, coenzyme A and glutathione (Marschner 1995). Sulfur also functions as an activator, cofactor, or regulator of enzymes and plays a role in the process of plant physiology. Elemental sulfur is an essential part of pherodoxyn, an iron and sulfur compound in the chloroplast. It is involved in an oxidoreduction reaction with electron transfer and nitrate reduction in the process of photosynthesis (Tisdalle et al., 1990).

Dolomite can increase chlorophyll because the supply of Mg from dolomite can increase the availability of soil Mg and Mg plant uptake (Suntoro 2002). Artificial chemical fertilizers supply certain nutrients in highly concentrated inorganic compounds and are easy to dissolve. Giving it repeatedly to plants can endanger the natural soil flora and fauna, bring in soil nutrient imbalances, cause water pollution, especially groundwater.

Organic fertilizers supply various nutrients, especially in the form of a low concentration of organic compounds that are not readily soluble, so they do not cause nutrient imbalances in soil, even improving the nutrient balance. The supply of organic matter can nourish the life of the soil flora and fauna, which in turn will improve and maintain soil productivity (Nuryani and Sutanto 2002).

A significant increase in biomass is a synergistic effect of organic extracts and chemical fertilizers in the soil. The supply of organic substrates through liquid organic fertilizer increases soil organisms' activity that plays a role in the decomposition of organic compounds and improves physical fertility (soil structure, aggregation, and aeration, increase water holding capacity), and increase nutrient availability (Reeves 1997). In such conditions, the supply of oxygen required for soil organisms' respiration is available. Therefore, soil quality improvement occurs in some phases and impacts *Sakti* chili biomass and tends to increase *Fantastic* chili biomass.

Chlorophyll content

Chlorophyll *a*

Fertilizer treatment affects chlorophyll-*a* content of two varieties of peppers (Table 2). Nutrients (nitrogen, phosphorus, magnesium, iron, manganese, potassium, calcium, sulfur) that accumulate in chemical fertilizer and organic fertilizer added to the manure treatment (1 kg/plant) + chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) + NPK *Mutiara* and manure (1 kg/plant) + chemical fertilizer (SP-36: KCl = 1:1) + liquid organic fertilizer can significantly increase the content of chlorophyll *a* in both varieties of chili. Chlorophyll-*a* is in the leaves of both varieties of peppers varies. This indicates that the physiological response of these two varieties of chili against a given nutrient supply is different. In general, it is said that the supply of nutrients from fertilizer can increase chlorophyll-*a* and both varieties of chili.

Table 2. Chlorophyll *a*, chlorophyll *b*, total chlorophyll at different fertilizer treatments given to two varieties of chili.

Variety	Chlorophyll content (mg/L)			
	P1	P2	P3	P4
Chlorophyll *a* (mg/L)				
Fantastic	2,52 a	5,99 ab	6,99 b	6,45 b
Sakti	2,65 a	6,29 b	7,48 bc	7,93 c
Chlorophyll *b* (mg/L)				
Fantastic	1,66 a	2,80 a	3,75 a	3,80 a
Sakti	1,68 a	3,23 ab	5,38 b	3,84 ab
Total chlorophyll (mg/L)				
Fantastic	4,17 a	8,78 ab	10,72 b	10,25 b
Sakti	4,33 a	9,51 b	13,31 b	11,31 b

Note: that noted the same on the same line mean there were not significantly different according to the DMRT test at P = 0.05. P1: Control, P2: Manure (2kg/plants), P3: Manure (1 kg/plant) Chemical Fertilizers (ZA: KCl: Sp-36 = 2: 1: 1) NPK *Mutiara*, P4: Fertilizer cage of chemical fertilizer (KCl: SP-36 = 1: 1) liquid organic fertilizer *Bathari Sri*

Chlorophyll *b*

Chlorophyll *b* functions as an antenna that gathers light and transfers it to the reaction center. The reaction center is composed of chlorophyll *a*. Light energy is converted into chemical energy in the reaction center, which can then reduce photosynthesis (Taiz and Zeiger 1991). Fertilizer treatment does not significantly affect chlorophyll *b* in two varieties of peppers (Table 2). This likely occurs because most of the chlorophyll is still at the stage of chlorophyll *a* (chlorophyll *a* of both varieties are proven to be significantly influenced by different fertilizer treatment) and has not become chlorophyll *b*, as we know that chlorophyll *a* is the precursor of chlorophyll *b* (Robinson 1980). Although fertilization treatments do not significantly affect the chlorophyll *b* content in both varieties of chili, there is a tendency that fertilization can increase the chlorophyll *b* both varieties of chili.

Total chlorophyll

The use of manure (1 kg/plant) + chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) NPK *Mutiara* shows the highest total chlorophyll content for the two varieties of chili because the artificial chemical fertilizers supply particular nutrients that are highly concentrated and easily soluble (N, P, K, Fe, Mg, S) that play a role in the formation of chlorophyll (Nuryani and Sutanto 2002). The control group had the lowest total chlorophyll content for both treatments because there were no nutrients from the outside. At the same time, the plant absorbed the available nutrients in the soil during the vegetative and early generative phases. Chlorophyll formation was disturbed because of the low availability and low nutrient absorption. Therefore, the chili chlorophyll content of the control group was relatively lower than other treatments.

The treatment of animal manure (1 kg/plant) + chemical fertilizer (SP-36: KCl = 1:1) liquid organic fertilizer is in the same group as the use of manure (1 kg/plant) of chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) NPK *Mutiara*. Nutrients (nitrogen, magnesium, iron, manganese), which accumulate in the chemical fertilizer added through fertilization, can increase the total chlorophyll content of leaves of *Sakti* chili (Table 2). This condition is possible because the elements of N, P, K, Mg, Fe, and S, chlorophyll-forming elements, are available and can be absorbed by plants.

After the analysis of the variant, it is known that fertilizer treatment doesn't significantly affect the total chlorophyll of *Fantastic* chili but has a significant effect on the total chlorophyll of *Sakti* chili (Table 2). The existence of a significant effect of fertilization on chlorophyll content indicates that the supply of nutrients (N, P, K, Mg, S) positively contributes to the process of leaf formation in *Sakti* chili. Meanwhile, the *Fantastic* chili nutrient supply provided through fertilization has not increased total leaf chlorophyll. This phenomenon indicates that the physiological response toward fertilization is not the same. Still, there is a tendency that fertilization can increase the total chlorophyll content of leaves. The manure and chemical fertilizer treatment (ZA: KCl: SP-36 = 2: 1: 1) NPK *Mutiara*) is always higher than any other treatments

(Table 2). The full content of nutrients in the fertilizer formulations can provide a stimulant for the increase in total chlorophyll content for both varieties of chili.

The addition of organic matter increases the leaf chlorophyll, and the increase will be higher if it is accompanied by adding the dolomite and KCl. The KCl fertilization increases the availability and uptake of phosphorus, while the potassium in the chloroplast acts as a guard to keep a high pH. Giving dolomite can increase chlorophyll because the supply of Mg from dolomite can increase the availability of soil Mg and Mg plant absorption. Magnesium plays a very important role in synthesizing chlorophyll (Suntoro 2002; Rahayu 2002; Santi 2002). Giving ZA, NPK *Mutiara* organic fertilizer can increase the chlorophyll because it can provide a combination of nitrogen and magnesium, which are known as elements that absolutely must be available for the formation of chlorophyll (Dwijoseputro 1986).

Nitrogen is closely related to the synthesis of chlorophyll (Salisbury and Ross 1992) and the synthesis of proteins and enzymes (Schaffer 1996). Rubisco enzyme acts as a catalyst in CO3 fixation that plants need for photosynthesis (Salisbury and Ross 1992; Schaffer 1996). Therefore, total nitrogen content in plants can influence the outcome of photosynthesis via photosynthetic enzymes and chlorophyll formation. In plants, nitrogen is initially in ammonia, and subsequent ammonia has been changed into glutamic acid, catalyzed by the enzyme glutamine synthetase (Harborne 1987). Glutamic acid is the base material in the biosynthesis of amino acids and nucleic acids (Nyakpa et al. 1988). Robinson (1980) called glutamic acid a precursor for the porphyrin ring in the formation of chlorophyll.

The chlorophyll formation mechanism begins with the formation of α aminolevulinic acid (ALA) (Stryer 2002). Formation of ALA through the stages of formation of glutamate through the glutamate-t-RNA, from glutamate and is converted into α-ketoglutarate semialdehyde then become the next-aldehyde with the enzyme transaminase or aminotransferase enzyme ALA formed (Bonner and Varner 1965; Krogman 1979). Of the two molecules involved, the enzyme ALA with dehydrated ALA will be composed porphobilinogen (PBG) containing pyrrole rings from four molecules of PBG to affect enzyme uroporphyrinogen III. Decarboxylation changes uroporphyrinogen III. Under aerobic conditions, by involving coproporphyrinogen decarboxylase enzyme, coproporphyrinogen III will then be formed into proporphyrinogen IX. Oxidation toward proporphyrinogen IX will result in proporphyrin IX, which does not have Mg; after joining Mg, then Mg protoporphyrin IX is formed. The addition of methyl groups on protoporphyrin IX Mg with the help of esterase protoporphyrin will form Mg porphyrin IX monomethyl ester. Next is the change of Mg porphyrin IX monomethyl ester into pro-chlorophyllide (Bonner and Varner 1965; Devlin 1983; Krogman 1979).

Change from protochlorophylide into chlorophyll takes place through the formation of protochlorophylide holochrome which binds to proteins that bind to ion 2H+, which will be given to the fourth ring which forms a protochlorophylide holochrome, which can then be turned into chlorophyll *a* by releasing holochrome and apoprotein (Mohr and Schopfer 1995).

If chlorophyll *a* with the help of an enzyme that catalyzes the esterification compounds chlorophyllase phytol can be formed into chlorophyll *a*. Meanwhile, leaf homogenate and thylakoid supply and leaves that is protected from light can convert chlorophyll *a* to chlorophyll *b*. Therefore, chlorophyll *a* can become a precursor of chlorophyll *b* (Robinson 1980).

Results of correlation analysis show that chlorophyll *a* has a positive relationship with chlorophyll *b* and total leaf chlorophyll and is positively related to plant fresh weight. An increase in chlorophyll *a* will increase the chlorophyll *b*, total chlorophyll and fresh weight of plant leaves. This can be easily understood because chlorophyll *a* is a precursor for chlorophyll *b*, while the chlorophyll *a* and *b* is the composition of total leaf chlorophyll, and also part of the plant fresh weight.

Leaf nitrogen content

Results of the research show that fertilizer treatments do not significantly influence the nitrogen content of leaves of two varieties of chili (Table 3). The results of this study support the findings of the previous research which claims a combination of urea and organic fertilizer does not affect the nitrogen and chlorophyll content of hermada grass (Sorghum bicolor) (Supriya and Soeharsono 2005). This is possible because, in all treatment combinations, the plant minimum requirement for nitrogen has been met.

Therefore, although the nitrogen content provided through fertilization is high but the plants absorb only a certain number of plants as needed.

Although the nitrogen content of leaves at different fertilizer treatment is not significant, but there is a tendency that the fertilizer treatment can increase the nitrogen content of leaves (Table 3). The tendency of leaf nitrogen content increase is a reflection of the increased nitrogen that can be absorbed by plants. The combination of ZA fertilizer, NPK *Mutiara*, and organic fertilizers cause an increase in leaf nitrogen content of plants. Nitrogen that is available in the soil that can be absorbed by plant roots is in the form of nitrate ions and ammonium. Both forms of nitrogen was obtained as a result of organic material decomposition. Nitrates that are absorbed by the roots and transferred into the upper parts of the plant is a result of the leaf transpiration process. Thus, nitrate assimilation in higher plants generally occurs in the leaves.

Table 3. Nitrogen content of leaves of two varieties of chilies on different fertilizer treatments.

Variety	Nitrogen content (mg/L)			
	P1	**P2**	**P3**	**P4**
Fantastic	3.64 a	3.46 a	3.85 a	4.12 a
Sakti	3.92 b	4.23 b	4.27 b	4.25 b

Note: letters which are same on the same line means not significantly different according to DMRT test at P = 0.05. P1: Control, P2: Manure (2 kg/plant), P3: Manure (1 kg/plant) + Chemical fertilizer (ZA: KCl: Sp-36 = 2: 1: 1) + NPK *Mutiara*, P4: manure + chemical fertilizer (KCl: SP-36 = 1: 1) + liquid organic fertilizer

The first step is the reduction of nitrate into ammonia. The second step, nitrite reaction which turns nitrite into nitrate that occurs to chlorophyll in the chloroplast. While the ammonia assimilation in most plants turns into glutamic acid, that serves as the base material in the biosynthesis of amino acids and nucleic acids (Harborne 1987; Nyakpa et al. 1988).

The tendency of increase in nitrogen content of plants can affect the photosynthesis either through chlorophyll content of photosynthetic enzyme. If the leaf nitrogen content increases, the photosynthate will increase; otherwise, if the leaf nitrogen content decreases, photosynthate formed is also low. That's because the elements of nitrogen will increase the green color of leaves, support stem and leaf growth (Marschner 1986).

Nitrogen is closely related to the synthesis of chlorophyll (Sallisbury and Ross 1992) and synthesis of proteins and enzymes (Schaffer 1996). Rubisco enzyme acts as a catalyst in the fixation of CO2 that plants need for photosynthesis (Salisbury and Ross 1992; Schaffer 1996).

Meanwhile, *Fantastic* chili leaf nitrogen content, manure treatment (1 kg/plant) + chemical fertilizer (SP-36: KCl = 1:1) + liquid organic fertilizer of 4.12%, followed by the use of manure + chemical fertilizer (ZA, SP-36, KCl = 2: 1: 1) + NPK *Mutiara* (3.85%), followed by the control group (3.64%) and the lowest manure application (2 kg/plant) amounted to 3.46% (Table 3).

The lower leaf nitrogen content in manure treatment than the control group at *Fantastic* chili was caused by the nutrients in the manure treatment cannot being absorbed optimally. This is related to the nature of the organic fertilizer that supplies a variety of nutrients, especially in the form of a low concentration of organic compounds that are not easily soluble (Nuryani and Sutanto 2002). The research results are consistent with research conducted by Salim (2006), which states that the type of soil management and organic fertilizer cannot increase the total nitrogen, K-leaf, and leaf nitrogen uptake.

CONCLUSION AND RECOMMENDATION

In *Fantastic* chili, a variety of fertilization treatment affects the biomass and chlorophyll *a*, but it does not affect chlorophyll *b*, total chlorophyll, and plant leaf nitrogen. The treatment of various kinds of fertilizers to *Sakti* chili effects on plant fresh weight, chlorophyll *a* and total chlorophyll and has no effect on dry weight, weight of fresh fruit, chlorophyll *b* and the nitrogen content of leaves. Researchers recommend using the formulation of fertilizers with manure + chemical fertilizer (SP-36: KCl = 1: 1) + liquid organic fertilizer as a new alternative to chili cultivation which is more economical and environmentally friendly.

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