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Correlation of Leaf NPK and Leaf Pigments of *Coleus atropurpureus* L. Benth during Vegetative and Generative Phases

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Abstract

Coleus atropurpureus L. Benth is a annual plant that has a distinctive leaf aroma and bitter taste. *C. atropurpureus* leaves contain phenolic compounds and antioxidants that can capture free radicals; free radicals play an important role in preventing various human diseases. A study was conducted to determine the correlation between leaf position (1st to 4th) at the vegetative and generative phases with leaf pigments, N, P, K, and total flavonoid concentrations. The results showed that leaf chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, anthocyanin, nitrogen, and total flavonoids were higher in the vegetative phase. Therefore, *C. atropurpureus* is better harvested in the vegetative phase, and the 2nd leaf position can be used as indicator for N, K, pigments and total flavonoid content.

Keywords: herbal plant, Lamiaceae, leaf position, nutrition, vegetative and generative phase

Introduction

Indonesians are well known to use medicinal plants as a treatment for health problem. One of the medicinal plants that has been widely used is jawer kotok (*Coleus atropurpureus* L. Benth). *Coleus atropurpureus* an annual herbaceous plant which can grow up to 100 cm tall (Wiart, 2006). *C. atropurpureus* grows upright and has branches with square rod shapes and jagged leaf edges (Figure 1). The length of the leaf stalk can reach 7.5 cm with an oval leaf shape 5-10 cm long. Flowers are purplish, white, or bluish on the terminal stalks with a shape like nails arranged 10-20 cm long. The colorful *C. atropurpureus* leaves make the plants to be used as ornamentals. The colors of the leaves differ with different types and cultivars. According to Osman (2013) *Coleus blumei* with purplish red and red to dark red leaves contains high phenolic levels, which indicates it is potential as medicinal plant.

Figure 1. *Coleus atropurpureus* in vegetative phase (left), and generative phase (right)

The genus of *Coleus* belongs to *Lamiaceae* or *Labiatae* family; many species from this family can be used in traditional medicine. *C. atropurpureus* leaves are usually used to overcome dermatitis, post partum, abdominal pain, coughing and muscles pains, particularly by people in West Java (Roosita et al., 2008). In addition, its uses to cure bronchitis, asthma, angina, digestive disorders, animal bites (Suva et al., 2016), for dengue fever and malaria drugs in Philippines (Gascon, 2011), for hemorrhoids, antioxidants and anti-tuberculosis (Ahmad and Massi, 2014) have been reported. *C. atropurpureus* contain saponins, flavonoids, alkaloids, polyphenols, quercetin and essential oils (Moektiwardoyo et al., 2011). The compounds which have antioxidant properties can capture free radicals and play an important role in preventing various chronic diseases

(Gross, 2004). One of antioxidants is flavonoid, which has been reported to inhibit proliferation of SP-C1 tongue cancer cells (Achmad et al., 2014). This study aims to determine whether or not different leaf positions contain different levels of secondary metabolite. The correlation between the position of leaves (1st to 4th) and the leaf nutrient content (NPK) and secondary metabolites during the vegetative and generative phases were also determined.

Materials and Methods

Plant Materials

Coleus atropurpureus leaves used are 5 MAP (months after planting) which planted in IPB University experimental station, Bogor, West Java, Indonesia. The fully open leaves from the 1st to the 4th position from the shoot tip were collected for analysis (figure2).

Figure 2. *Coleus atropurpureus* showing leaf 1 to 4 for nutrient and pigment analysis

Leaf Nutrient and Pigment Analysis

Analysis of nutrient levels of N, P, and K was carried out in the Testing Laboratory of the Department of Agronomy and Horticulture, IPB University. Total N analysis used Kjeldahl method, P was determined using a UV-1800 UV-VIS spectrophotometer, and K analysis used AAS (Atomic Absorption Spectrophotometry). Analysis of chlorophyll, carotenoid, anthocyanin and total flavonoids was carried out in the Postharvest Laboratory of the Department of Agronomy and Horticulture, IPB University. Analysis of chlorophyll, carotenoid and anthocyanin used Sims and Gamon (2002) method with the following protocol: 200 mg of fresh leaves were weighed, mashed with 2 ml acetic solution, centrifuged (6000 rpm, 10 minutes), 1 ml supernatant was added 3 ml of acetic acid then measured with wavelengths of 470, 537, 647 and 663 nm. The total level of flavonoids was analyzed using the method of Chang et al (2002): 10 mg quercetin was dissolved in 80% ethanol then diluted to 25, 50 and 100 µg/ml. The diluted 0.5 ml solution was mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of potassium acetate 1M and 2.8 ml of distilled water. This solution was incubated at room temperature for 30 minutes, absorbance was measured at 415 nm. Blanks were made by replacing the amount of aluminum chloride with distilled water. An extract in 0.5 ml of ethanol was reacted with aluminum chloride to determine the flavonoid concentration. According to Aziz (2015) production of bioactive compounds can be carried out with the following method: bioactive compound production = leaf dry weight (g per plant) x concentration of leaf bioactive compounds (%).

Data Analysis

Data were analyzed using R for t-student test and SAS 9.4 for Pearson correlation test.

Results and Discussion

Leaf Pigment at The Vegetative and Generative Phase

The leaf clorophyll a and clorophyll b at the vegetative phases is in Figure 3, and the highest content are in the 3rd leaf. However, chlorophyll a and chlorophyll b levels in each leaf position showed fluctuating results. Leaves at positions 1st, 2nd, and 4th had 21.61%, 23.80%, and 22.37% lower chlorophyll a than the 3rd leaf, whereas leaves at the 1st, 2nd, and 4th had 20.16%, 22.62%, and 20.98% lower chlorophyll b than the 3rd leaf. Leaves at position 1st, 2nd, and 4th have total chlorophyll levels which were 21.14%, 23.40%, and 21.92% lower than the 3rd leaf. The carotenoid levels in the 1st and 3rd leaves were not significantly different from each other, but were significantly higher than the 2nd and 4th leaves. The 2nd leaf showed a markedly lower carotenoid level of 12.97% and the 4th leaf showed a markedly lower carotenoid level of from the 1st leaf position.

The level of anthocyanin in the 1st and 2nd leaf was not significantly different, but was significantly higher than 3rd and 4th leaves. At the 4th leaf position there was an increasing anthocyanin levels of 10.81%. The level of leaf pigment at the generative phase is described in Figure 4, and it shows fluctuating results. Chlorophyll a and carotenoid levels showed the highest results at the 4th leaf position. The 3rd position leaves have anthocyanin levels that are significantly different from the 1st, 3rd and 4th leaf.

Leaf NPK Levels at The Vegetative and Generative Phase

The levels of N and P at the vegetative and generative phases were not significantly different from those in the generative stage (Figure 5) but N levels in the vegetative phase was 0.22% higher. The highest P at the vegetative and generative phase was found in the 1st leaf. Nutrient K from the 1st and 2nd leaf at the generative phase is greater than the vegetative phase. N levels of the 2nd and 3rd leaves, as well as the 3rd and the 4th leaves were not significantly different. Leaf P decreases along the leaf position and this occurs in the vegetative and the generative phases. In the generative phase, the levels of P of the 1st, 2nd , 3rd leaf was 0.08%, 0.03%, and 0.003% higher than the 4th leaf. The 1st and 2nd leaves had higher potassium levels than the 3rd and 4th leaves, but the levels of the 3rd and 4th leaves were similar. In the vegetative phase, the levels of n decreased with from the 1st to 4th leaves, the levels of the 1st, 2nd , 3rd were 0.82%, 0.30%, and 0.24% higher than in the 4th leaf. Similar trend was noticed in the generative phase, where the levels of N of the 1st , 2nd , 3rd leaves were 0.51%, 0.24%, and 0.07% higher the levels of N of the 4th leaf. In addition, the nutrient P levels in the generative phase was higher 0.27% compared to the vegetative phase.

The first leaf had the highest K level in the vegetative and generative phase, and K levels was 2.40% higher in the generative compared to the vegetative phase. K level of the 1st , 2nd , and 3rd leaves were 0.27%, 0.13%, and 0.18% higher than K level of 4th leaf. In the generative phase, there was a decreasing K levels from the position of the 1st to 4th leaves; K levels of the 1st , 2nd , 3rd leaves were 1.48%, 0.90%, and 0.03% higher than the 4th leaf.

Total Flavonoid Concentration and Total Flavonoid Content

The highest total flavonoids concentration in the vegetative and generative phases were found at the 1st leaf (Figure 6). In the vegetative phase, total flavonoids concentration decreased according to the position 1st to 4th leaves; the levels of the 1st , 2nd , and 3rd leaves were 49.00%, 16.50%, and 7.76% higher than the 4th leaf. Similar trend was noticed during the generative phase; total flavonoids of the 1st , 2nd, and 3rd leaves were at 25.58%, 5.92%, and 3.23% higher than the 4th leaf.

There was a fluctuating total flavonoid content in the vegetative phase from 1st to 4th leaf but the 4th leaf had highest total flavonoid content in both vegetative and generative phase (Figure 7). The total flavonoids content was obtained from multiplication of total flavonoids concentration to leaf dry weight. The 3rd and 4th leaves were larger and heavier than other leaves. The total flavonoids content of the 1st, 2nd, and 3th leaves were 0.39%, 0.77% and 0.25% lower than the 4th leaf in vegetative phase. Total flavonoid content decreased from the 1st to 4th leaves at the generative phase, which was 0.41%, 0.14%, and 0.17% lower

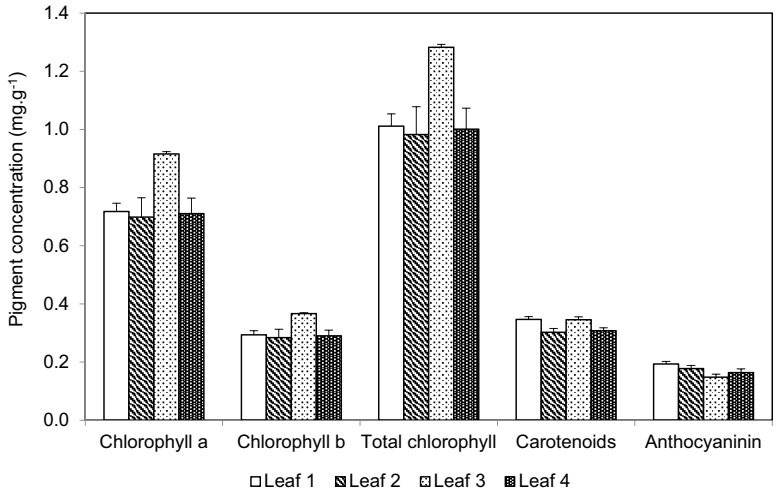

Figure 3. Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyanin levels of *Coleus atropurpureus* leaf at the vegetative phase

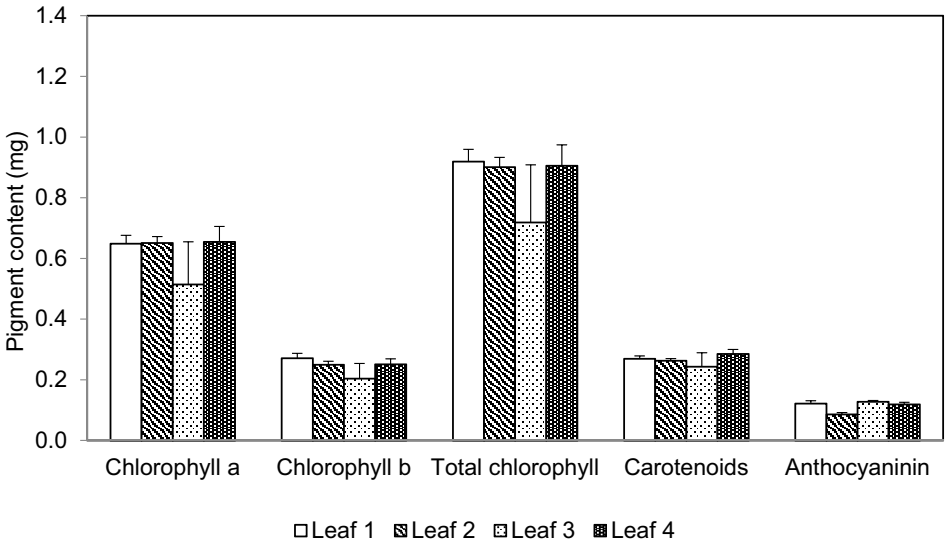

Figure 4. Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, and anthocyaninin levels of *Coleus atropurpureus* leaf at the generative phase

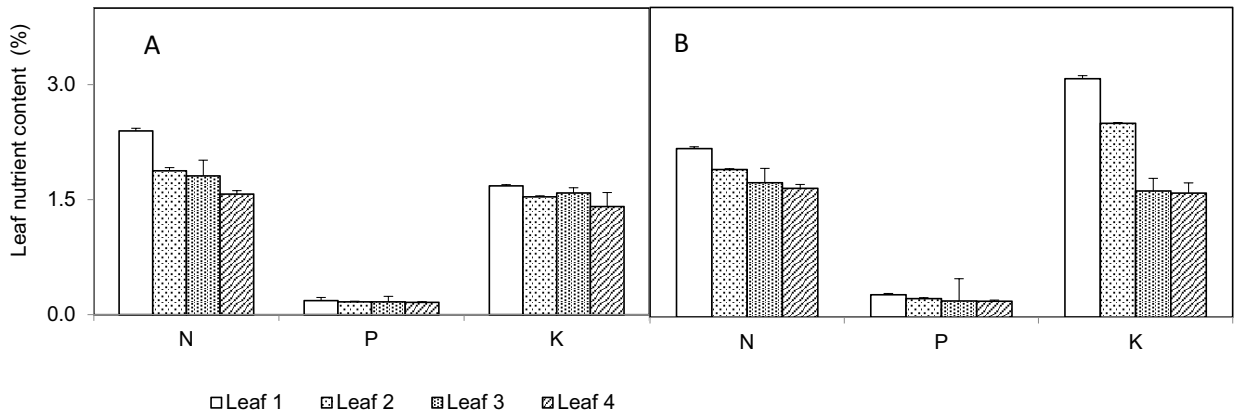

Figure 5. The leaf nitrogen, phosphorus and potassium of *Coleus atropurpureus* leaves at the vegetative and generative phase

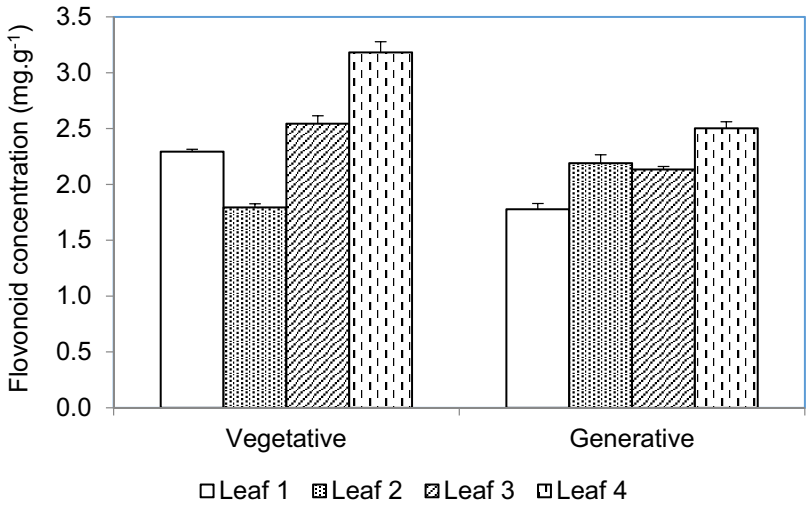

Figure 6. Total flavonoid concentration of *Coleus atropurpureus* leaves at the vegetative and generative phase

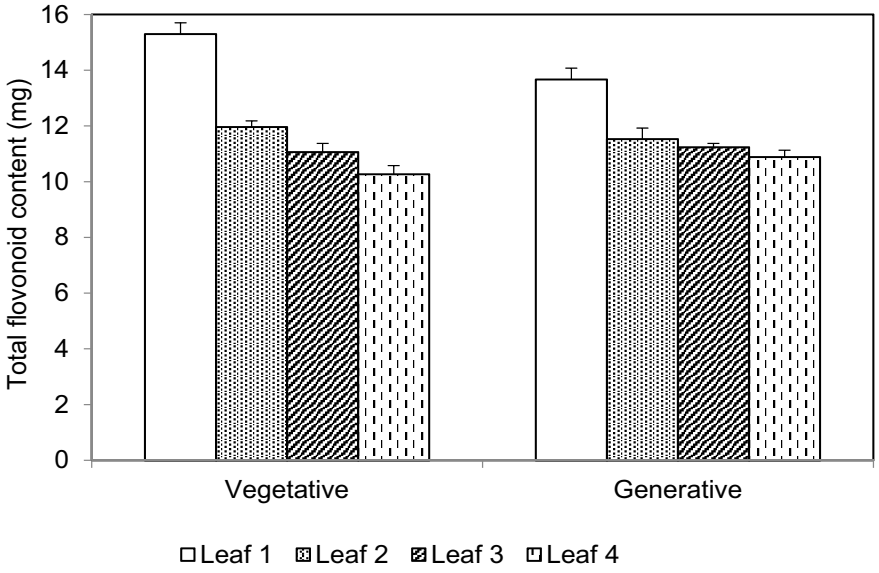

Figure 7. The total flavonoid content of *Coleus atropurpureus* leaves at the vegetative and generative phases

than the 4th leaf. The values of the total flavonoids at the vegetative phase was 27.18% higher compared to those at the generative phase.

Table 1 showed the correlation between leaf NPK with leaf chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin, carotenoids and total flavonoid of the first to fourth leaves at the vegetative phase. N levels were positively correlated with chlorophyll a, total chlorophyll, carotenoid levels, at the 1st, 2nd and 3rd leaves positions, and positively correlated with anthocyanin and total flavonoid levels and levels in the 1st, 2nd, and 4th leaf positions. In the 2nd, 3rd, and 4th leaf N has a positive correlation with chlorophyll b. In the 1st, 2nd, and 3rd leaf P has a positive correlation with chlorophyll a, chlorophyll b, carotenoids, total chlorophyll. In the 1st, 2nd and 3rd leaf K was positively correlated with chlorophyll a, chlorophyll b, carotenoids, and positively correlated with anthocyanin in the 2nd and 4th leaf. A positive correlation between K and total content of flavonoids were recorded in all leaf positions.

Table 2 showed the correlation at the generative phase. N was positively correlated with levels of chlorophyll a, chlorophyll b, carotenoid, and total

Table 1. Pearson correlation values between NPK of the first to the fourth leaf with leaf chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin, carotenoids and total flavonoid (mg.g^{-1}) at the vegetative phase

Leaf position	Nutrient	Chlorophyll a	Chlorophyll b	Carotenoid	Anthocyanin	Total chlorophyll	Total flavonoid
1	N	0.305	-0.088	0.305	0.324	0.178	0.362
	P	0.909	0.999*	0.909	-0.978	0.955	0.882
	K	0.920	0.999*	0.920	-0.972	0.964	0.895
2	N	0.891	0.873	0.935	0.461	0.886	0.958
	P	0.218	0.255	0.112	-0.974	0.229	-0.515
	K	0.475	0.440	0.565	0.884	0.464	0.950
3	N	0.691	0.143	0.981	-0.029	0.560	-0.916
	P	0.652	0.091	0.970	0.023	0.516	-0.935
	K	0.891	0.989	0.467	-0.966	0.954	0.122
4	N	-0.113	0.184	-0.997*	0.272	-0.034	0.152
	P	-0.625	-0.368	-0.884	0.743	-0.56	0.656
	K	-0.494	-0.216	-0.947	0.628	-0.423	0.528

Note: *significant differences according to Pearson corellation test at α= 5%.

Table 2. Pearson correlation values between NPK of the first to fourth leaf with leaf chlorophyll a, chlorophyll b, total chlorophyll, anthocyanin, carotenoids and total flavonoid ($mg.g^1$) at the generative phase

Leaf position	Nutrient	Chlorophyll a	Chlorophyll b	Carotenoid	Anthocyanin	Total chlorophyll	Total flavonoid
1	N	-0.945	-0.365	-0.981	0.762	-0.797	0.478
	P	-0.756	-0.989	-0.321	-0.179	-0.922	0.999*
	K	-0.378	-0.949	0.142	-0.605	-0.647	0.903
2	N	-0.948	-0.892	-0.204	0.140	-0.931	-0.999*
	P	0.663	0.546	-0.313	-0.616	0.623	0.891
	K	0.064	0.209	0.894	0.993	0.115	-0.306
3	N	-0.937	-0.931	-0.971	0.218	-0.936	0.971
	P	0.721	0.732	0.636	-0.974	0.724	-0.206
	K	0.999*	0.999*	0.990	-0.566	0.999*	-0.812
4	N	0.636	0.690	0.554	-0.410	0.650	0.999*
	P	-0.982	-0.993	-0.958	0.899	-0.986	-0.761
	K	-0.972	-0.986	-0.943	0.875	-0.976	-0.793

Note:* significant differences according to Pearson corellation test at α= 5%

chlorophyll of the 4th leaf , but positively correlated with anthocyanin of the 1st, 2nd, and 3rd leaf Leaf N also positively correlated with levels and the total content of flavonoids in the 1st and 3rd leaf. Leaf P has a positive correlation with the levels of chlorophyll a, chlorophyll b, total chlorophyll in the position of the 2nd and 3rd leaves, while with the carotenoids in the position of the 3rd leaf. P nutrient content was positively correlated with anthocyanin at the 4th leaf position, while positively correlated with the levels and total flavonoid content in the 1st and 2nd leaf positions. Nutrient content K was positively correlated with levels of chlorophyll a, chlorophyll b at the 2nd and 3rd leaf position, while positively correlated with carotenoids at the 1st, 2nd, and 3rd leaf positions. K nutrient levels were positively correlated with anthocyanin in leaf position 2nd and 4th, while positively correlated with total chlorophyll at the 3rd leaf position. Nutrient K has a positive correlation with the level and total content of flavonoids in the 1st leaf position.

Discussion

The presence of pigments in the form of chlorophyll a and chlorophyll b play a role in absorbing solar radiation during photosynthesis. Photochemical processes can act to release electrons, so that light energy is converted into chemical energy. This level of chlorophyll can affect photosynthesis (Richardson et al., 2002). Photosynthesis rates are directly proportional to the concentration of photosynthetic pigments. Therefore, the position of leaf that close to apex will have a low pigment concentration. Marschner (2012) reported that in mature leaves ~ 15% of the volume of all cells is occupied by chloroplasts, cytoplasm and cell walls while the remainder is by vacuoles (85%). Therefore, in mature leaves the levels of bioactive compounds had higher levels of anthocyanin, chlorophyll and flavonoids.

In croton concentration of chlorophyll a, chlorophyll b and total chlorophyll were found to be higher in older leaves, and the carotenoid level in the generative phase is lower than the vegetative phase (Gogahu et al., 2016). Research by Tjhia et al., (2018) reported that the levels of carotenoid in *Vernonia amygdalina* Del at the generative phase was higher than that the vegetative phase. Chlorophyll activity plays a role in the process of organogenesis which can affect the generative phase (Simova et al., 2001). All pigment levels in the vegetative phase are higher than the generative phase. Similarly for leaf weights. The level of anthocyanin in the generative phase decreases because in the early phase of this development anthocyanins are required to carry out photoprotection. Anthocyanin is needed because at this stage, chlorophyll cannot develop properly to absorb excessive sunlight. Decreased anthocyanin levels in the generative phase indicate that anthocyanin function might have been replaced by the presence of carotenoids (Hughes et al., 2007).

Higher nitrogen levels affect chlorophyll levels in each phase because nitrogen is one of the important component of chlorophyll (Marschner, 2012). Photosynthesis has a positive relationship with the growth process of all parts of the plant (Diem et al.,

2000). In cotton, increasing photosynthesis rate is affected by increasing CO_2 uptake, while CO_2 uptake is affected by ion concentration including K^+, NO_3^-, PO_4^- (Longstreth et al., 1980). With a decrease in the concentration of nitrogen and phosphorus, plants experience stress and will respond with an increase in anthocyanin. Anthocyanin functions as an antioxidant which will free radicals when stress occurs (Scott, 1999).

Plants have young leaf parts that act as sinks and adult leaves act as sources (Marschner, 2012). The position of the leaf can indicate the direction of the nutrient translocation path and the water for the plant sink towards the source. Usually, the young leaves have higher nutrient levels and become strong sinks. Nitrogen concentration in both vegetative and generative phases decreased from the 1st to 4th leaf position, and potassium concentration decreased in the generative phase but the level is still high.

A decrease in nitrogen in the tissue causes a decrease in protein and chlorophyll content. Munawar (2011) reported that nitrogen plays an important role for plants. Nitrogen is involved in the synthesis and transfer of energy, plant growth, improves leaf quality, seed and fruit production, and plays a role in the preparation of amino acids, proteins, chlorophyll, nucleic acids and co-enzymes.

There is a relationship between the availability of nutrients and the accumulation of flavonols; reduction of nitrogen will increase flavonol levels. But in the availability of high nitrogen, phosphate reduction can facilitate the formation of flavonols. Nitrogen deficiency in tomato plants produces accumulation of flavonol in adult leaves. Conversely, when phosphorus deficiency causes accumulation of flavonol at the beginning of fruit ripening (Stewart et al., 2001). Karimuna et al. (2015) reported the total flavonoids of *Murraya paniculata* leaves were negatively correlated with potassium at different leaf ages and positions. Potassium concentrations can be categorized very high (3.59-4.10%), phosphorus was high (0.28-0.29%) or very high (0.33-0.35%).

Conclusion

Coleus atropurpureus leaf chlorophyll a, chlorophyll b, carotenoids, anthocyanin, total chlorophyll, nitrogen, total levels of flavonoids and total flavonoid content at the vegetative phase were higher than those at generative phase. The indicator leaf at the vegetative phase which have positive correlations with pigment and total flavonoids are the second leaf, whereas all leaf positions at the generative phase do not have correlations with the leaf NPK, leaf pigments, and leaf total flavonoids.

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