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Accumulation of Selected Plant nutrients of Sweet basil (*Ocimum basilicum* L.) as Influenced by Different Nitrogen Fertilizer Rates and leaves Maturity

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Abstract

Sweet basil (*Ocimum basilicum* L.) is a medicinal plant species belonging to the family Lamiaceae. The essential oil of cultivated sweet basil is used for flavoring in food and beverages. Demand for sweet basil is increasing annually, justifying that an effort should be made to increase its production. Since the economic value of this herb comes from its leaves, effort has been made to increase the number of leaves in order to upsurge the amount of phytochemicals in leaves. Application of nitrogen promotes leaves production in sweet basil which will increase the production of phytochemicals as well. The present study was carried out to determine the Phytochemical contents of *Ocimum basilicum* collected from different leaves maturity. In this study, seeds of sweet basils were cultivated under 50% shade in three different plots with the application of 100, 150 and 200 kg/ha nitrogen fertilizer. The plants were harvested in different stages and taken to laboratory for further analysis. Plants from each treatment were divided into three sections; young leaves, intermediate leaves and mature leaves in order to study the effects of different leaf growth stages on the amount of plant nutrients. Between three tested groups, application of 150 kg/ha nitrogen fertilizer had the highest accumulation of N, P, K (Nitrogen

3.08 %, Phosphorus 0.5% and potassium 2.46%) at the mature leaves in the upper part of the plant.

Keywords**:** Plant - section, plant-sections, fertilizer, sweet basil

1. Introduction

The quality of sweet basil lies on the amount of phytochemical accumulated in the leaves. Sweet basil has been reported to have more than 200 phytochemical compounds like as monoterpenes, terpinolene, flavonoids, phenolic acids and steroids (Marvat *et al*., 2011). Application of nitrogen influenced the production of phytochemical contents in plants (Munene *et al*., 2017). Nitrogen is well known to influence the primary metabolites in plants. According to Chen et al. (2011), the pathway for production of secondary metabolites is correlated with the pathway in producing the primary metabolites. Wu *et al.* (2013) reported that higher amount of nitrogen was found in older leaves compared to young leaves in Limanium bicolor plant. In the case for plants that grow vertically, leaves position at low section were higher in the amount of nitrogen in comparison to leaves position at the top section of plants (Trapani and Hall, 1996). Other than the position of the leaves, the cultural practices plays an important role in influencing the accumulation of biochemical contents in the leaves. Based on the report presented by Nguyen and Niemeyer. (2008), the amount of fertilizer influences the accumulation of biochemical contents. Thus, the aim of this experiment was to explore the effects of nitrogen application at field on accumulation of phytochemicals at different plant's sections of sweet basil.

2. Materials and methods

In this experiment 100, 150, and 200 kg/ha nitrogen fertilizer under 50% shade treatments were used. Each plant from each treatment was divided into three sections which were top, intermediate and low sections based on its measurement from soil surface (15 cm, 25 cm and 35 cm, respectively). Different mature leaves were harvested and tested for the quality parameters such as extraction yield percentage, nitrogen percentage, phosphorus percentage and potassium percentage. The experiment was designed in factorial Randomized Complete Block Design

(3X3) with four replications and each replication consisted of five samples. Data were subjected to Analysis of Variance (ANOVA) using Statistical Analysis System (SAS) and means were compared by Least Significant Difference (LSD) at 5%.

2.1. Plant extraction

The fresh leaves were harvested and placed in an oven at 55°C. Prior of placing the leaves, the oven was pre-heated. Once the leaves were fully dried (reached constant weight), the leaves were grinded with a grinder (Brand: Kniematica). Total of 5 g of powder form sample was mixed with distilled water (100 mL). Solutions were refluxed at 65°C for 2 hours, then cooled (at room temperature) and filtered through filter paper (No. 1, Whitman). Mixture was then placed in rotary evaporator (EYELA Miyagi, Japan) under reduced pressure at temperature of 90°C about 90 seconds. Mixture was freeze dried and dried extracts were kept at -20°C for further analysis (Ghasemzadeh et al., 2016).

2.2. Extraction yield

The extraction yield was measured to obtain the specific weight of total extract yield. The end product obtained from subtopic 4.2.1 was placed inside the flask and weight by using digital balance ((Model: FX 1200, Japan).) to obtain the weight of extract (W1). The yield of extraction was calculated using the following formula:

Extraction yield= W1/W2 X 100

Where

W1 = extract (sample after evaporated from rotary evaporator or known as crude extract)

W2 = Initial weight of sample before mixing with distilled water (5 g).

2.3. Plant nutrients analysis

Plant nutrient analysis was determined by measuring nitrogen, phosphorus and potassium elements inside the leaves of sweet basil. Wet digestion was used in this method. Dried leaf 0.25 g was placed in digestion tube and mixed with 5 ml of sulphuric acid (50%) and kept for 24 hours. After 24 hours, the sample mixture was heated using digestion block at 285°C for 45 minutes. Then, a total of 2 ml of hydrogen peroxide was added into solution with interval time of 45 minute for three times until the solution become clear. Final product of digestion was diluted using distilled water and filtered using Whatman filter paper No. 42. Lastly, the volume of solution was made up into 100 ml using volumetric flask. Finally, about 10 ml solution was used for the analyzing of total N, P (using auto analyzer) and K (using atomic absorption spectrophotometer).

3. Result and Discussion

3.1. Extraction yield

The extraction yield was found to be affected by the application of different nitrogen fertilizer at field and the position of leaves on the stem. Table 1 presented the interaction of both factors on the extraction yield of sweet basil at $p<0.05$. In the 2-ways analysis, it was found that leaves position at low-section (mature leaves) were having the highest extraction yield percentage which was 13%. The extraction yield was reduced to 12.3% for intermediate-section leaves and to 11.3% for upper-section leaves. In the combined effect, leaves positioned at top sections for plants treated with 100 kg/ha nitrogen fertilizer having similar extraction yield with leaves positioned at intermediate sections. Increasing the rate of nitrogen to 150 and 200 kg/ha N fertilizer showed increased in the amount of extraction yield in the intermediate leaves section which was 13%. Besides, it was found that the extraction yield was similar in all leaves at low sections and not affected by different nitrogen rates (13-14%). Thus, it can be stated that application of nitrogen fertilizer on sweet basil determined the pattern of accumulation of extraction yield in the young and intermediate leaves.

Table 1. The interaction effect of different levels of nitrogen and leaves maturity on extraction yield (%) of sweet basil.

Nitrogen (kg / ha)	Extraction yield %			
	Young leaves	**Intermediate leaves**	**Mature leaves**	**Mean**
100	11.50c	11.50c	14.00a	12.33A
150	12.00b	13.00ab	12.50ab	12.5AB
200	10.50d	12.50ab	12.50ab	11.83C
Mean	11.33C	12.33B	13A	

Means with the same letter in the same column are significantly different at $P \leq 0.05$ based on LSD.

According to Wu *et al.* (2013), nitrogen fertilizer takes a central role in the system of plant metabolism. Thus, this study has revealed that the amount of nitrogen alters the amount of extraction yield. Besides, Rao et al. (2007) mentioned that elevated nitrogen availability (doses over 100 kg/h nitrogen) resulted in higher plant extraction yield in basil plant. Nevertheless, the effect of nitrogen fertilizer on the leaves of a plant as a whole has not been studied in depth. By comparison, different nitrogen gradients were created along the vines of plants grown at low nitrogen fertilizer concentrations because newly developed leaves contained high nitrogen levels, but nitrogen fertilizer decreased with the progressing of the age of leaves.

3.2. Nitrogen

Accumulation of nitrogen on sweet basil leaves in response to different sections and nitrogen fertilizer rates is presented in Table 2. In two-ways statistical analysis, the highest nitrogen percentage was recorded from young leaves (3.03%). In this section, the application of urea was not giving any significant effect on the accumulation of the nitrogen. Same pattern was found in intermediate section, where the accumulation of nitrogen was not significant with each other (3%).

Table 2. The interaction effect of different levels of nitrogen and leaves maturity of plant on nitrogen% of sweet basil.

Nitrogen	**Sections**			
(kg / ha)	**Young leaves**	**Intermediate leaves**	**Mature leaves**	**Mean**
100	3.01ab	2.93b	2.84b	2.92B
150	3.08a	3.03ab	3.01ab	3.04A
200	3.00ab	3.00ab	2.67c	2.89B
Mean	3.03A	2.98B	2.84B	

Effect of different levels of nitrogen fertilizer and leaves maturity on plant nitrogen percentage of sweet basil plant. Means with the same letter in the same column are significantly different at $P \leq 0.05$ based on LSD.

The lowest accumulation was found in low section leaves with the application of urea at 200 kg/h N fertilizer which was 2.67%. As there was only slight difference in terms of nitrogen percentage so it can be stated that the amount of nitrogen in sweet basil leaves were almost the same despite of the leave position and urea application. It has been reported that the minimum nitrogen in basil plant is around 2.4% (Nurznska *et al.*, 2011). In this study, the amount of nitrogen in sweet basil leaves was 0.6% higher compared to report by Nurznska *et al.* (2011). Such differences were due to the absorption efficiency of nitrogen from the soil. Furthermore, an increase in the percentage of nitrogen may be due to nitrogen fertilization which urea was used as the source of nitrogen in this study. The result is similar with other researcher who found that the application of nitrogen at 150 kg/ha N led to increase the concentration of nitrogen 4% in plant tissue in sweet basil (Hamblin, 1986). Report from Escudero and Mediavilla. (2003), showed that nitrogen content was found to be higher in young leaves compare to mature leaves, mean young leaves take more nitrogen. It is because of the growth and therefore need more nitrogen for the formation of new cells in the leaves (Wang *et al.*, 2012).

3.3 Phosphorus

The amount of phosphorus at different section of sweet basil plant in response to nitrogen rates is presented in Figure 1. There is no interaction between nitrogen rates and plant sections. It was found that young leaves have the highest accumulation of phosphorus which was 0.5%, followed by intermediate and mature leaves which were 0.4 and 0.2%, respectively. Besides, the application of urea seems to influence the accumulation of phosphorus in the leaves. Urea application at 150 kg/h N showed accumulation of 0.45% of phosphorus, meanwhile, the amount of phosphorus dropped to 0.3% at 200 kg/h N application.

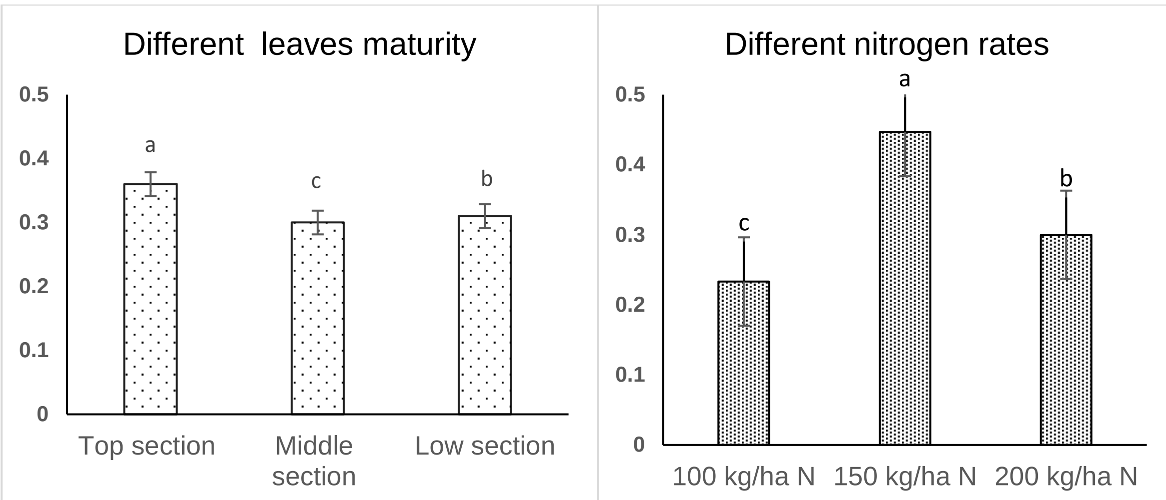

Figure 1: Effect of different leaves maturity and nitrogen rates on sweet basil Phosphorus percentage.

As all plants were grown in the same type of media, therefore it is assumed that the amount of phosphorus present in the leaves is the amount that being absorbed by the plants from the media. Phosphorus plays an important role in lateral root branching and root morphology (Razaq *et al.*, 2017). Pan *et al.* (2016) explained that nitrogen is essential in plant metabolism system. Other than the accumulation of phosphorus in the leaves, Pan *et al*. (2016) emphasized that applying nitrogen to plants also improved phosphorus use efficiency. Furthermore, high amount of phosphorus which was 0.53% was found in with urea application at 150 kg/ ha N (Biesiada, and Kus 2010).

3.3 Potassium

The amount of potassium in leaves is presented in Table 3. The highest percentage of potassium 2.46% was recorded at young leaves of plant. And the lowest Potassium percentage was recorded 2.22% at mature leaves of plant. However; It was found that those treated with 200 kg/ha N fertilizer were having higher nitrogen percentage compared to 100kg/ha N and 150kg/ha N in all leaves of basil plant. Besides, there was interaction between section of plant and levels of nitrogen. Furthermore, application of 100 kg/ha N and 150 kg/ha N were same in all leaves of plants.

Table 3. The interaction effect of different levels of nitrogen and leaves maturity of plant on potassium% of sweet basil.

Nitrogen	Sections			
(kg / ha)	**Young leaves**	**Intermediate leaves**	**Mature leaves**	**Mean**
100	2.46a	2.27c	2.24d	2.32B
150	2.31c	2.40b	2.22d	2.31B
200	2.33c	2.43b	2.31c	2.36A
Mean	2.36A	2.36A	2.25B	

Effect of different levels of nitrogen and leaves maturity of plant on potassium percentage of sweet basil plant. Means with the same letter in the same column are significantly different at $P \leq 0.05$ based on LSD.

However, Nurzynska-Wierdak. (2011), reported that, nitrogen significantly affected the concentration of potassium in basil herb. Potassium least effected this component in herbs. Besides, the increasing accumulation of potassium have contributed to protein elevation and total concentration of nitrogen in basil herb. According to Tsay *et al.* (2011), interaction between nitrogen and potassium is a very sophisticated phenomenon and occurs at many levels of plant metabolism, having an exclusionary effect on the absorption mechanisms between these elements.

4. Conclusion

Different leaves' maturity and rate of nitrogen application have significantly increased the phytochemicals (nutrients) (Extraction yield, nitrogen percentage, phosphorus percentage and potassium percentage) accumulation in sweet basil plant. It was observed that the application of

150 kg N/ ha fertilizer and 100 kg N/ ha fertilizer influences the accumulation of elements especially at mature leaves of plants, followed by the intermediate leaves and young leaves of plants.

It is likely that, mature leaves of plant provided more suitable environment for the growth of sweet basil plant. Furthermore, nitrogen levels also showed a variation in the response of plant elements. Nevertheless, nitrogen levels also showed a variation in the response of plant phytochemical accumulations in which 150 kg N/ ha fertilizer was found to be the best fertilizer levels at young leaves of plant for elements accumulation of sweet basil. Future studies are recommended to run the experiment of different plant density and nitrogen application.

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