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Nutritional diagnosis of nitrogen and phosphorus in *Ocimum basilicum* L. plants grown under macronutrient applications

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Abstract

The objective of this work was to assess the nutritional status of basil (*Ocimum basilicum* L.) plants grown under application of macronutrients, in a Typic Hapludult. The experiment was conducted in Cruz das Almas, BA, Brazil, in a greenhouse. The treatments were based on a Baconian matrix statistical arrangement, in which one of the nutrients is supplied in different quantities, whereas the other nutrients are maintained at the reference rates. Six nutrients, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) were evaluated at three different rates. Two additional treatments were used (reference rates; and without nutrient applications), totaling 20 treatments. A completely randomized experimental design with five replications was used, totaling 100 experimental units. Plant material was collected 50 days after treatment applications and dried in an oven for 72 hours. Approximately 0.1 g of dry weight of leaves, stems, and roots were subjected to acid digestion in a mixture of concentrated sulfuric acid and hydrogen peroxide. The digested material was diluted in 100 mL of distilled water to obtain the extract for nutritional diagnosis. The optimal maximum N and P accumulations in basil leaves for high plant performances were 51 and 3.0 g kg^{-1}, respectively. The estimated rates for each nutrient to provide the optimal total N accumulation in basil plants were: N = 235.61, P = 91.27, K = 175, S = 41.98 (mg dm^{-3}), Ca = 0.97, and Mg = 0.36 (cmolc dm^{-3}).

Keywords: Basil, plant mineral nutrition, soil fertilization

Introduction

Basil (*Ocimum basilicum* L.) is a medicinal plant species that is native to tropical Asia; it presents good adaptation to hot and mild climate regions (LORENZI & MATOS, 2008). It is a species from the family Lamiaceae that stands out for its spicy, aromatic, and medicinal characteristics (MARQUES et al., 2015). This species has raised significant economic interest due to the use of its leaves in cooking, as well as medicinal applications, such as headache, fever, diarrhea, cough treatments (MIRANDA et al., 2016). Consequently, extraction of basil essential oil has been intensified due to its useful compounds, such as linalool, which is its main compound and highly demanded by the pharmaceutical industry (VLASE et al., 2014).

The high importance of *A. basilicum* as a medicinal plant highlights the need to increase its production, which requires considering several essential factors to make the species to express its genetic potential, including soil moisture, relief, luminosity, irrigation, climate conditions, and soil fertility (MEIRA et al., 2012; CALIXTO, 2000).

The management of essential nutrients is one of the the most important agronomic practice to improve plant performance (PAL et al., 2016), as supplying appropriate quantities of these elements can maximize agricultural production and, consequently, result in a satisfactory economic return (MCGRATH et al., 2014).

Nitrogen (N) is a primary macronutrient and the most limiting nutrient to crops. Its importance is highlighted by its participation in metabolic pathways and formation of amino acids, proteins, enzymes, ATP, NADH, NADPH, and chlorophylls, as well as in synthesis of vitamins and hormones and other compounds (Taiz et al., 2017).

Phosphorus (P) can be found in organic and inorganic forms in the soil and has a direct effect on plant metabolism; thus, it is essential for root system

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development, flowering, fertilization, and grain formation and maturation (CARMO et al., 2014). Phosphorus deficiency hinders the plant growth and decreases leaf area, number of leaves, and stem diameter (TAIZ et al., 2017).

Nutritional diagnosis in plants is important because it provides information on deficiency of essential nutrients and, consequently, susceptibility to diseases and environmental stress (ELMER et al., 2014). Several plant physiological mechanisms depend on macro and micronutrients and their beneficial processes, such as production of photoassimilates, enzyme activation, cell respiration, synthesis of secondary metabolites, lignin, phenols, phytoalexins, and other defense substances (TAIZ et al., 2017).

In this sense, the objective of this work was to evaluate N and P accumulation in basil plants grown under application of macronutrients, using different nutritional arrangements.

Material and Methods

The experiment was conducted in a greenhouse at the experimental area of the Federal University of Reconcavo da Bahia (UFRB), in Cruz das Almas, Bahia (BA), Brazil. Soil samples from the 0-20 cm layer were collected in the experimental area and sent to a laboratory for chemical characterization (**Table 1**). The soil of the area was classified as a Typic Hapludult (Argissolo distrofico; SANTOS et al., 2018).

The treatments were based on Baconian matrix statistical arrangement (TURRENT, 1979), consisting of supplying one of the nutrients in different quantities, whereas the other nutrients are maintained at reference rates. The treatments consisted of six nutrients (nitrogen, phosphorus, potassium, calcium, magnesium, and sulfur) at three different rates, with two additional treatments, consisting of reference rates (FERREIRA et al., 2016; MATOS et al., 2016; ABREU et al., 2013; LIMA et al., 2013) and absence of nutrient application, totaling 20 treatments. A completely randomized experimental design was used, with five replications, totaling 100 experimental units. The treatments were designed considering that the rates stablished for the nutrients remains constant when the rates of one nutrient varies (**Table 2**).

The substrate used consisted of 6 kg of soil in each plastic pot (capacity of 6 dm³). Calcium and magnesium were applied to the substrates, using a homogenized mixture of $CaCO_3$ and $MgCO_3$ at the rates established for the treatments. Subsequently, the substrate pots were subjected to incubation for 45 days to ensure the effectiveness of fertilizer solubilization; the water content was maintained at approximately 60% of field capacity, according to Marques et al., (2015).

The other nutrients were then applied at seedling transplanting, according to the established rates (Table 2). The N application was split; the second half was applied 30 days after the first application. The salts used were: CH_4N_2O and $(NH_4)_2SO_4$ for N and S; KCl and KH_2PO_4 for K and P.

The sowing of basil seeds (Feltrin®) was carried out in polyethylene pots (capacity of 0.3 dm³), using three seeds per pot. The most uniform seedlings, with 10 cm in height and two pairs of fully expanded leaves, were selected at 21 days after sowing and then transplanted into the permanent pots.

Leaves, stems, and roots were collected from the basil plants 50 days after transplanting, placed in individual paper bags, and dried in a forced air circulation oven at 45±2 °C until constant weight. Subsequently, the dried materials were ground in a Wiley mill, standardized in size using a 20-mesh sieve, and placed in plastic bags. Approximately 0.1 g of dry masses of leaves, stems, and roots were subjected to acid digestion in a mixture of 3.5 mL of concentrate sulfuric acid (H_2SO_4) and 3

Table 1. Chemical characterization of the 0-20 cm layer of the Typic Hapludult used for growing basil (Ocimum basilicum L.) seedlings

pH	P	K	Ca^{2+}	Mg^{2+}	Al^{3+}	H+Al	SB	CECe	CECt	OM	BS
H_2O	mg dm^{-3}					$cmol_c$ dm^{-3}					%
5.6	0	7.82	0.8	0.5	0	1.5	1.32	1.32	2.82	0.96	46.81

pH in water; P and K = Mehlich 1 method; Ca^{2+}, Mg^{2+} and Al^{3+} = KCl 1 mol L^{-1}; H + Al = calcium acetate 0.5 mol L^{-1} (pH 7.0); SB = sum of exchangeable bases; CECe = effective cation exchange capacity (pH 7.0); CECt = total cation exchange capacity; OM = organic C × 1.724 (Walkley-Black method); BS = base saturation index.

Table 2. Treatments used, based on Baconian matrix, with the respective rates of nutrients applied to the substrate for growing basil (*Ocimum basilicum* L.) plants

Treatments	Units	Treatments	Units
Reference rate*	mg dm^{-3} and $cmol_c$ dm^{-3}	Ca= 0.5**	$cmol_c$ dm^{-3}
Without fertilizer application	-	Ca= 1.5**	
N = 75 **	mg dm^{-3}	Ca= 2.0**	
N = 225**		Mg= 0.2**	
N = 300**		Mg= 0.6**	
P = 30**		Mg= 0.8**	
P = 90**		S= 20**	mg dm^{-3}
P = 120**		S= 60**	
K = 75**		S= 80**	
K = 225**		S= 80**	
K = 300**			

*Reference rates: N = 150 mg dm^{-3}; P = 60 mg dm^{-3}; K = 150 mg dm^{-3}; Ca = 1 $cmol_c$ dm^{-3}; Mg = 0.4 $cmol_c$ dm^{-3}; S = 40 mg dm^{-3}. ** In each treatment, only the indicated nutrient (N) had varied rates, whereas the other nutrients remained at the reference rates.

mL of hydrogen peroxide (H_2O_2) at 30% (JONES, 2001). The digested material was diluted in 100 mL of distilled water to obtain the extract for analyses of nitrogen N and P. The N contents were determined by phenol-hypochlorite reaction (WEATHERBURN, 1967) and P contents were determined by the spectrophotometric molybdovanadate method (FAITHFULL, 2002).

The data were subjected to analyses of variance and regression, using the statistical program SISVAR. The response curves to the application of each nutrient resulted in regression models and the coefficients were tested based on the mean square residuals (ANOVA) at 1% and 5% probability levels (FERREIRA, 2011).

Results and Discussion

The means found denoted significant effects of the treatments on the accumulation of N in the different parts of basil plants as a function of increases in the rates of the evaluated macronutrients. The regression analysis enabled to estimate the optimal rate for each evaluated nutrient while keeping the others nutrients at the reference rates. Thus, the estimated rates of each nutrient for N accumulation in basil leaves were: N = 165.27, P = 83.75, K = 172.56, S = 46.5 (mg dm^{-3}), Ca = 0.91, and Mg = 0.37 ($cmol_c$ dm^{-3}). N accumulations higher than 45 g kg^{-1} were found from these rates onwards (**Table 3**), highlighting K and S rates, which resulted in the highest N accumulations (51.45 and 51.58 g kg^{-1} respectively) in basil leaves.

A synergistic effect was found between N accumulation in the basil leaves and K and S rates. Considering that N is a primary macronutrient that participates in several metabolic activities, it is required in larger quantities by most plant species (FERNANDES, et al., 2018). The N accumulations found in the present study are similar to those found by Marques et al., (2015), who evaluated nutrient contents in basil leaves using different K rates in successive crops.

Regarding N accumulations in stems and roots, higher results were found for the highest N rates: 11.74 and 16.41 g kg^{-1}, respectively (Table 3). Considering the regression analysis for N accumulation in stems, the estimated rates for each nutrient were: N = 300, P = 46.51, K = 184.21, S = 30.71 (mg dm^{-3}), Ca = 2.0, and Mg = 0.4 ($cmol_c$ dm^{-3}). N and Ca rates were 100% higher than those recommended for the crop (reference rates).

Regarding N accumulation in roots, the estimated rates for each nutrient were: N = 300, P = 120, K = 187.5, S = 27.5 (mg dm^{-3}), Ca = 0.73, and Mg = 0.4 ($cmol_c$ dm^{-3}). These estimates indicated that basil plants had higher N accumulation in roots than in stems, with a linear response to increases in N rates; thus, the highest N accumulation was connected to the highest tested N

Table 3. Estimates of N accumulation in basil (*Ocimum basilicum* L.) plants as a function of application of increasing rates of N, P, K, S (mg dm^{-3}), Ca, and Mg ($cmol_c$ dm^{-3})

Variables (g kg^{-1})	Treatments	Equation	R² (%)	Estimated rate
N in leaves	N	Y**: 26.34+0.238x-0.00072x²	69.71	165.28
	P	Y**: 26.93+0.536x-0.0032x²	82.22	83.75
	K	Y**: 27.04+0.283x-0.00082x²	96.12	172.56
	Ca	Y**: 30.0+38.05x-20.09x²	73.71	0.91
	Mg	Y**: 29.65+85.50x-116.75x²	61.21	0.37
	S	Y**: 27.8+1.023x-0.011x²	96.16	46.5
N in stems	N	Y**: 3.64+0.027x	70.49	300
	P	Y**: 5.08+0.04x-0.00043x²	90.93	46.51
	K	Y*: 5.02+0.014x-0.000038x²	98.26	184.21
	Ca	Y**: 4.72+2.744x	80.44	2.0
	Mg	Y**: 7.37+12.97x-20.56x²	21.69	0.31
	S	Y**: 7.0+0.129x-0.0021x²	29.79	30.71
N in roots	N	Y**: 7.56+0.0295x	89.8	300
	P	Y**: 7.79+0.061x	91.0	120
	K	Y**: 8.07+0.045x-0.00012x²	38.49	187.5
	Ca	Y**: 8.44+5.586x-3.844x²	95.69	0.73
	Mg	Y**: 8.31+13.36x-16.5x²	56.19	0.40
	S	Y**:8.88+0.055x-0.001x²	74.89	27.5
Total N accumulation	N	Y**: 40.6+0.213x-0.000452x²	80.48	235.62
	P	Y**:40.76+0.575x-0.00315x²	87.17	91.27
	K	Y**: 40.14+0.343x-0.00098x²	94.65	175
	Ca	Y**: 43.22+46.22x-23.86x²	87.26	0.97
	Mg	Y**: 45.34+111.84x-153.82x²	73.91	0.36
	S	Y**: 43.69+1.209x-0.0144x²	92.95	41.98

** and * = significant at 1% and 5% probability, respectively.

rate. It can be attributed to the possibility that a large part of the absorbed N was metabolized and allocated as reserves in leaves and roots.

The effect of the treatments on total N accumulation in basil plants was significant at 1% probability for all evaluated macronutrients (Table 3). This result was similar to that found for N accumulation in leaves, mainly for K and S rates, which resulted in the highest estimated total N accumulations: 70.15 and 69.06 g kg^{-1}, respectively. The estimated rates for each nutrient were: N = 235.61, P = 91.27, K = 175, S = 41.98 (mg dm^{-3}), Ca = 0.97, and Mg = 0.36 ($cmol_c$ dm^{-3}).

Regarding P in basil leaves, a mean accumulation of 2.6 g kg^{-1} (**Table 4**) was found for the following estimated nutrient rates: N = 300, P = 120, K = 150, S = 80 (mg dm^{-3}), Ca = 1.02, and Mg = 0.43 ($cmol_c$ dm^{-3}). The increases in N, P, and S rates resulted in a linear response, i.e., the quantity of P accumulated in the leaves significantly increased as the N, P, and S rates were increased. The highest estimated P accumulation in leaves as a function of P rates was 3.82 g kg^{-1}.

However, the highest accumulation of these nutrients in leaf tissues does not always result in high yields for the species. Lima et al. (2020) evaluated the growth and yield of basil plants as a function of application of macronutrient fertilizers and found the highest leaf biomass productions for the estimated N and P rates of 250 and 80.66 dm^{-3}, respectively. Thus, the critical contents of N and P in leaves for maximum biomass production should be between 40.84 and 49.4 g kg^{-1} and between 2.54 and 3.01 g kg^{-1}, respectively. This phenomenon is explained by the nutritional requirements of each plant species, with an optimal concentration for each nutrient, which implies that nutrient rates below the optimal range can cause decreased plant growth and yield (TAIZ, et al. 2017).

The effect of the treatments on P accumulation in stems was significant at 1% probability for all evaluated macronutrients, except for S. The estimated rates for each nutrient were: N = 117.65, P = 50.63, K = 156.25, S = 20 (mg dm^{-3}), Ca = 0.45, and Mg = 0.8 ($cmol_c$ dm^{-3}). The basil plants presented higher accumulation of P in stems than in leaves from these estimated rates onwards, highlighting the Mg rates, which resulted in increases P in stems up to approximately 3.54 g kg^{-1}, denoting a probable synergism between these nutrients.

The mean P accumulation found in the roots (1.51 g kg^{-1}) showed that the roots accumulate lower P than leaves and stems. The basil plants presented, on average, 72% more P in leaves than in roots. The estimated rates for each nutrient (N = 173.91, P: 40.65, K: 160, S: 39.28 mg dm^{-3}, Ca: 0.92, and Mg: 0.48 $cmol_c$ dm^{-3}) were different from the reference rates for the crop.

The effect of the treatments on P total accumulation in basil plants was significant for

Table 4. Estimates of P accumulation in basil (*Ocimum basilicum* L.) plants as a function of application of increasing rates of N, P, K, S (mg dm^{-3}), Ca, and Mg ($cmol_c$ dm^{-3})

Variables (g kg^{-1})	Treatments	Equation	R^2 (%)	Rate estimated
P in leaves	N	Y**: 1.54+0.004x	94.97	300
	P	Y**: 1.34+0.0207x	92.19	120
	K	Y**: 1.50+0.006x-0.00002x²	81.14	150
	Ca	Y**: 1.48+1.29x-0.632x²	95.65	1.02
	Mg	Y**: 1.53+2.66x-3.07x²	70.73	0.43
	S	Y**: 1.68+0.0089x	68.06	80
P in stems	N	Y**: 2.83+0.004x-0.000017x²	45.57	117.65
	P	Y**: 2.75+0.008x-0.000079x²	69.37	50.63
	K	Y*: 2.64+0.005x-0.000016x²	69.36	156.25
	Ca	Y**: 2.74+0.45x-0.499x²	76.15	0.45
	Mg	Y**: 2.68+1.07x	96.74	0.8
	S	Y: 2.79-0.00008x		20
P in roots	N	Y*: 0.95+0.008x-0.000023x²	97.16	173.91
	P	Y**: 0.95+0.01x-0.000123x²	73.39	40.65
	K	Y*: 1.01+0.008x-0.000025x²	91.10	160
	Ca	Y**: 1.11+1.24x-0.671x²	67.42	0.92
	Mg	Y**: 0.97+2.79x-3.02x²	89.54	0.48
	S	Y**:0.91+0.022x-0.00028x²	42.45	39.28
Total P accumulation	N	Y**: 5.77+0.005x	62.69	300
	P	Y**:5.41+0.021x	94.03	120
	K	Y**: 5.16+0.0204x-0.000062x²	98.81	164.52
	Ca	Y**: 5.33+2.98x-1.80x²	79.21	0.83
	Mg	Y**: 5.67+1.65x	65.96	0.8
	S	Y**: 5.11+0.059x-0.00063x²	77.71	48.82

** and * = significant at 1% and 5% probability, respectively.

all evaluated macronutrients. The highest total P accumulations (7.27 and 7.93 g kg^{-1}) were found for the estimated N and P rates, respectively. A linear response was found for increasing N, P, and Mg rates. The estimated rates for each nutrient were: N = 300, P = 120, K = 164.52, S = 48.82 (mg dm^{-3}), Ca = 0.83, and Mg = 0.8 ($cmol_c$ dm^{-3}).

The results found in the present study are important information for the control and monitoring of the nutritional status of basil plants, which can be used as reference standards in agricultural crops for this species.

Conclusions

The optimal maximum N and P accumulations in basil leaves found for high plant performances were 51 and 3.0 g kg^{-1}, respectively.

The highest N accumulations in basil plants were found for the following estimated rates for each evaluated nutrient: N = 235.61, P = 91.27, K = 175, S = 41.98 (mg dm^{-3}), Ca = 0.97, and Mg = 0.36 ($cmol_c$ dm^{-3}).

The highest total P accumulation found in basil plants grown in a Typic Hapludult was 7.93 g kg^{-1}.

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