

Analysis of macro and micro nutrients in six substrates and leaves for vanilla cultivation (*Vanilla planifolia* G. Jackson)

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ABSTRACT

Objective: To analyze the physicochemical properties and nutritional content in six substrates and the foliar content of macro and micronutrients in leaves of five phenological stages in vanilla.

Design/methodology/approach: Six substrates were evaluated, in which physicochemical properties and the content of total and available macro and micro nutrients were determined. The foliar content in leaves of five phenological stages in vanilla was also determined. The statistical analysis had a completely randomized design, the substrates and phenological stages were evaluated separately, with two repetitions per treatment.

Results: The substrates analyzed resulted in total amounts of macro and micro nutrients acceptable for good development of vanilla plants and, considering the foliar contents of the leaves, adequate management of the nutrition of the vanilla crop can be achieved.

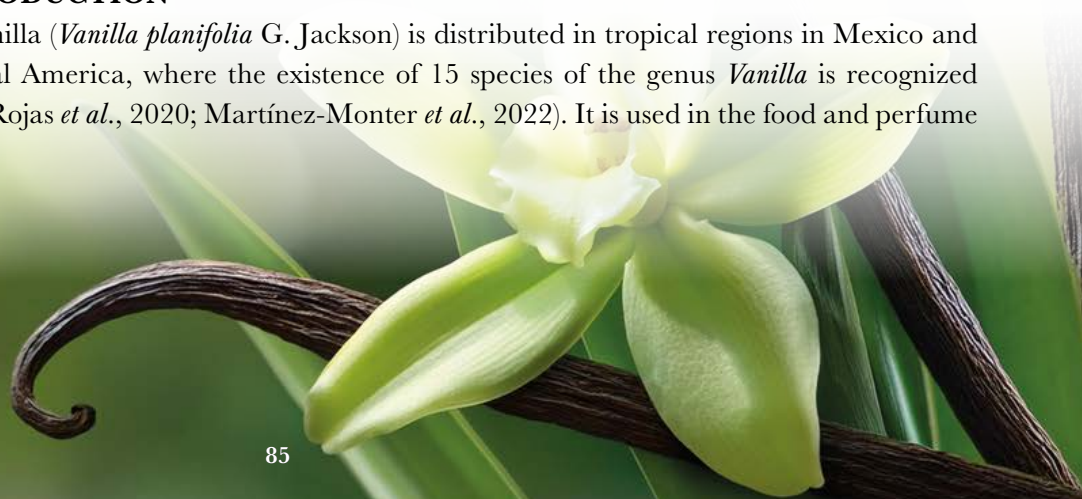
Limitations on study/implications: The physicochemical analyses of the substrates present total amounts of nutrients for the proper development of vanilla plants. However, their availability must be improved.

Findings/conclusions: The foliar contents of macro and micro nutrients in vanilla leaves were different in the stages of plant development, with K being the one with the highest foliar content followed by N, P, Ca and Mg. Considering these foliar contents and the total and available nutritional contents in the substrates can contribute to adequate nutrition planning in vanilla cultivation.

Keywords: macronutrients, micronutrients, physicochemical properties, *Vanilla planifolia* G. Jackson.

INTRODUCTION

Vanilla (*Vanilla planifolia* G. Jackson) is distributed in tropical regions in Mexico and Central America, where the existence of 15 species of the genus *Vanilla* is recognized (Luis-Rojas *et al.*, 2020; Martínez-Monter *et al.*, 2022). It is used in the food and perfume



industry to obtain flavoring and aromatic substances, as well as in the field of medicine, because it has anti-mutagenic and anti-cancer properties (Arya *et al.*, 2021; Ramos-Castellá and Iglesias-Andreu, 2022). Vanilla presents a root system which develops superficially on substrates with large amounts of organic matter in decomposition (woody and leafy residues), product of pruning of trees and crops such as banana (Martínez-Monter *et al.*, 2022).

Nowadays, conventional commercial substrates are used, although these inputs increase the production cost (Santillán *et al.*, 2018). In addition to this, the substrates used generate environmental problems due to residue elimination; therefore, an alternative with low environmental impact is sought, such as local organic materials that are stable, of good quality, safe and which allow the adequate development of the crop. This will depend on the local agroindustry, which provides a diverse range of materials such as coconut fiber, wood chips, sawdust, and sugarcane bagasse (González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022).

Vanilla producers in Mexico use plant residues in their substrates, but there is no control over the nutrition levels, which is one of the important aspects to obtain good production, in addition to climate factors, substrate, management and age of the crop (de la Cruz *et al.*, 2014; González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022). Foliar analysis is a useful method to understand the nutritional status of a crop, since it assesses indirectly the fertility of the substrate or any culture medium. The substrates used currently for vanilla cultivation are elaborated empirically, and there are few studies related to nutrition of this crop in Mexico. Therefore, the objective of this study was to evaluate the concentration of total and available macro and micro nutrients in six different substrates, as well as the foliar content of macro and micro nutrients in leaves of five phenological stages of the vanilla crop. The hypothesis is that at least one of the substrates satisfies the nutritional needs in each of the phenological stages of the vanilla plant.

MATERIALS AND METHODS

Study site

The research was conducted in May, 2022, in the municipality of Tenampulco, Puebla, Mexico, located on geographic coordinates 20° 11' 52.23" N and 97° 22' 06.09" W, at an altitude of 240 m; with semi-warm climate and frequent summer rains (INEGI, 2005).

Vanilla substrates

In the elaboration of substrates for vanilla, available local materials from the study region were used, such as cattle manure, soil from the forest, banana leaves and stems (*Musa paradisiaca* L.), bamboo leaves (*Bambusa vulgaris* Vittata), and sawdust; the proportions of each component are presented in Table 1. The substrates chosen were organic materials, with the aim of not generating environmental problems and seeking stability and good quality in them for good growth of vanilla (González-Chávez *et al.*, 2018; Martínez-Monter *et al.*, 2022). For the analysis of total and available macro and micro nutrients, a sample of 1 kg was taken from each of the substrates, dried at room temperature, and sieved through a 2 mm mesh.

Table 1. Materials and proportions used for vanilla substrates.

Material	Sub 1	Sub 2	Sub 3	Sub 4	Sub 5	Sub 6
	%					
Manure	50	40	30	30	20	30
Soil	15	30	30	40	20	10
Leaf 1	25	20	30	20	50	50
Leaf 2	5	5	5	5	5	5
Sawdust	5	5	5	5	5	5

Sub=substrate; Leaf 1=banana leaf; Leaf 2=bamboo leaf.

Foliar analysis

The leaf samples were obtained from five phenological stages of vanilla, days after sowing (DAS): 1) Growth stage, from 0 to 240 DAS, 2) Before production, from 241 to 360 DAS, 3) In production, from 361 to 600 DAS, 4) Before flowering, from 601 to 660 DAS, and 5) During flowering, from 661 to 730 DAS. Sampling was conducted in 10 plants and three leaves were taken from each, for a total of 30 leaves for each of the phenological stages. The leaf samples were washed, dried, ground, sieved, and stored in plastic bags for their study (Silva and Uchida, 2000). Both analyses were carried out in the Laboratory of the Centro de Investigaciones en Ciencias Microbiológicas (ICUAP), Puebla.

The physicochemical characterization was conducted in each of the substrates: pH with method AS-02, electric conductivity (AS-18), organic matter (AS-07 from Walkley and Black), and apparent density with the AS-03 method of the NOM-021-RECNAT-2000 (SEMARNAT, 2000). With the AS-25 method of the NOM-021-RECNAT-2000, the total N of the substrates was evaluated. Meanwhile, the total macro-elements (P, K) and micro-elements (Na, Ca, Mg, Fe, Mn, Zn and Cu) in the substrate were determined with the calcination method. The available P in the substrate was determined with the ammonium fluoride method. Meanwhile, the available elements in the substrate (K, Na, Ca, Mg, Fe, Mn, Zn, and Cu) were determined with the ammonium acetate extraction method, and the total leaf elements (P, K, Na, Ca, Mg, Fe, Mn, Zn and Cu) with the microwave digestion method (SEMARNAT, 2000).

Statistical analysis

Six treatments were obtained for the substrates and 5 treatments for the phenological stages of vanilla. The substrates and the leaf samples had two repetitions per treatment. The experimental unit was 34.7 g for the substrates (physicochemical characteristics, total and available elements); and 0.4 g for the phenological stages. The normal distribution of the observations was carried out with a significance of $p \leq 0.05$, and they were analyzed with a completely randomized design. Analysis of variance (ANOVA) was conducted with the data from the substrates, and another ANOVA with the phenological stages of vanilla. In each ANOVA, Tukey's means comparison ($p \leq 0.05$) was added. The analyses were carried out with the statistical software SAS[®] version 9.0.

RESULTS AND DISCUSSION

The analysis of variance for the physicochemical variables, the content of macro and micro elements in the substrates and leaves were highly significant ($p \leq 0.05$), with the exception of the physicochemical AD variable and nitrogen in foliar analysis.

The physicochemical analysis showed that the pH in the six substrates ranged between 9.50 and 8.28 (Table 2), being higher in substrate five. In general, the six substrates can be considered to be alkaline, and these results differ from what was reported by Reyes-López (2021), due to the materials used for substrate manufacturing. On the other hand, Ortiz *et al.* (2022) mentioned that the pH in the organic substrate should range from 5.5 to 6.8. Likewise, Barbaro *et al.* (2019) recommend for a substrate to have a slightly acidic pH, in order for nutrients to be available. On the other hand, Montoya-Jasso *et al.* (2021) mentioned that alkaline composts are useful because they contain lower proportion of exchangeable hydrogen ions, high contents of calcium, magnesium and sodium. However, considering the NMX-FF-109-SCFI-2007, the pH of substrates 1, 2, 3, and 4 fall within the range established by that norm, since it establishes a range of 5.5 to 8.5.

Regarding the EC, it was found that it varied between 7.76 ds m^{-1} in substrate two to 3.38 ds m^{-1} in substrate three, and these differences were statistically significant ($p \leq 0.05$). Merino and Rodríguez (2018) mention that, in a substrate of optimal range, EC should range between 1.2 and 3.5 ds m^{-1} , considering that this range fulfills these values only in substrate three. In OM, there are also significant differences ($p \leq 0.05$), with substrate one being the one with highest amount of OM of 26.02%, and substrate three with the lowest value of OM of 17.88%, due to the amount of manure used. However, in most of the substrates in this study there are low percentages of OM, except for substrate one, since an adequate percentage in substrates is from 25 to 30%. However, the NMX-FF-109-SCFI-2007 establishes a range of 20 to 50%, with which most of the substrates would be within that range. No statistically significant differences were found in AD. However, these values can be considered high; Montoya-Jasso *et al.* (2021) mention that the AD in substrates should be lower than 0.60 Mg m^{-3} .

Regarding the analysis of total and available macro elements N, P, and K, they were statistically different ($p \leq 0.05$), with substrate four being the one that presented the highest

Table 2. Physicochemical variables evaluated in the substrates used.

Treatments	pH	CE (ds m^{-1})	MO (%)	Da (Mg cm^{-3})
Substrate 1	8.31 b	6.63 bc	26.02 a	0.73 a
Substrate 2	8.45 b	7.76 a	20.48 bc	0.75 a
Substrate 3	8.28 b	3.38 e	17.88 c	0.77 a
Substrate 4	8.28 b	5.47 d	20.59 bc	0.75 a
Substrate 5	9.50 a	7.14 ab	24.05 ab	0.76 a
Substrate 6	9.01 ab	6.19 c	20.49 bc	0.73 a
DMS	0.97	0.65	3.94	0.07

pH=hydrogen potential; CE=electric conductivity; MO=organic matter; Da=apparent density. Values with the same letter in the lines are statistically equal according to Tukey's means test with $p \leq 0.05$. DMS=Minimum Significant Difference.

values of nitrogen, because more soil was added, which possibly already contained nitrogen; on the other hand, substrate three presented the lowest values of N because it was the one where less amount of soil and banana leaves were used. The nitrogen content varies depending on the components of the substrate, since manure can increase the amount of nitrogen between 0.53 and 1.25% (Aguñaga-Bravo *et al.*, 2020). On the other hand, the NMX-FF-109-SCFI-2007 establishes a total nitrogen range of 1 to 4%, where the six substrates comply with this norm.

Regarding phosphorus and potassium, the total and available values presented broad differences, possibly due to the pH found in the substrates, which being alkaline decrease the availability of nutrients. Potassium presented higher availability in all the substrates compared to phosphorus (Table 3). González-Chávez *et al.* (2018) proposed a range of 10 to 1000 ppm of total phosphorus in substrates. The composition of substrates determined the total and available amount of macro elements, since potassium presented higher total and available amount in substrates 5 and 6 because they have a higher amount of banana leaves (Table 1).

When it comes to total and available micro elements, similar to macro elements, values were observed with broad difference (Table 4), in relation to Na, Ca and Mg; Ca was the one that presented highest availability, followed by Mg and Na. Likewise, the highest values of available Ca and Mg was found in substrate five, which is due to the banana leaf, since it has higher percentage in the composition of this substrate. From these results, it can be inferred that banana leaf can be an element to consider in the manufacture of organic substrates, since it contributes a good amount of potassium, calcium, magnesium and copper.

Regarding Fe, Mn, Zn and Cu, no defined pattern was observed when it comes to total and available amount, because there is no coincidence between these amounts in the same substrate. However, it is observed that Mn presents higher values of availability in the six substrates, followed by Zn, Fe and Cu. In this sense, Gayosso-Rodríguez *et al.* (2018) mention that the optimal levels of Mn in organic substrates should be higher than 0.02 ppm.

Table 3. Total and available macro elements present in the substrates.

Treat.	Nitrogen	Phos. (T)	Phos. (D)	Pota. (T)	Pota. (D)
	ppm				
Subs. 1	7250 a	5297.62 c	14.46 b	6950.00 d	2123.15 c
Subs. 2	7950 a	6344.95 a	5.12 f	8150.00 c	2283.20 b
Subs. 3	4800 b	4261.90 d	13.21 c	6150.00 e	1583.55 d
Subs. 4	8250 a	4202.17 d	19.28 a	6350.00 e	2162.25 c
Subs. 5	7400 a	5702.38 b	11.66 d	10500.00 b	2895.35 a
Subs. 6	7150 a	5225.98 c	9.04 e	11050.00 a	2805.45 a
DMS	1194	206.53	0.63	344.68	92.98

Treat.=Treatment; Phos.=Phosphorus; Pota.=Potassium; T=total element; D=available element; ppm=parts per million; Subs.=Substrate. Values with the same letter within rows are equal according to Tukey's test at $p \leq 0.05$. DMS=Mínima Significativa Diferencia.

Table 4. Total and available micro elements in substrates.

Treat.	ppm													
	Na (T)	Na (D)	Ca (T)	Ca (D)	Mg (T)	Mg (D)	Fe (T)	Fe (D)	Mn (T)	Mn (D)	Zn (T)	Zn (D)	Cu (T)	Cu (D)
Sub 1	5450.0 a	290.0 b	51500.0 c	7414.4 ab	15550.0 b	1822.9 bc	2972.0 f	16.6 d	210.9 d	51.9 d	140.5 c	27.5 b	24.2 b	7.6 c
Sub 2	4600.0 b	380.0 a	55000.0 b	6612.9 bc	18650.0 a	2673.6 ab	3441.5 e	14.3 e	165.1 e	23.2 f	193.0 a	23.3 c	27.7 a	6.9 d
Sub 3	2350.0 d	171.5 d	40500.0 e	6612.9 bc	9750.0 f	2552.1 ab	4519.0 b	23.4 a	291.1 a	79.1 a	122.5 e	21.5 cd	15.4 e	9.5 a
Sub 4	3600.0 c	208.0 cd	44500.0 d	7815.2 a	12050.0 e	1093.8 c	4321.5 c	21.4 b	280.6 b	49.3 e	131.5 d	29.6 a	19.1 d	6.6 d
Sub 5	5200.0 a	238.0 c	54500.0 bc	8416.6 a	13700.0 c	3524.3 a	3896.0 d	17.2 d	254.3 c	57.2 c	164.0 b	20.7 d	27.1 a	9.1 b
Sub 6	3550.0 c	202.0 cd	59500.0 a	6212.1 c	13100.0 d	3524.3 a	4781.0 a	20.6 c	289.0 a	59.6 b	161.5 b	18.3 e	21.4 c	7.6 c
DMS	444.9	46.9	3446.8	1029.7	513.8	1279.6	9.3	0.6	5.4	0.7	5.1	0.9	0.6	0.4

Treat=Treatments; Sub=substrate; Na=sodium; Ca=calcium; Mg=magnesium; ; Sub = Substrate; Fe = iron; Mn = manganese; Zn = zinc; Cu = copper; T=total element; A=available element; ppm =parts per million. Values with the same letter in the lines are equal according to Tukey's test at p≤0.05. DMS=Minimum Significant Difference.

In some studies of substrates for vanilla cultivation, Martínez-Monter *et al.* (2022), when elaborating bokashi with forest soil, sawdust, corn stubble, sugarcane bagasse, bovine manure, carbon, ash, yeast and unrefined brown sugar, found higher amount of calcium (6.88 g Kg^{-1}), followed by nitrogen (1.58 Kg^{-1}), and potassium (1.39 Kg^{-1}); likewise, with pH of 8.4 and EC of 3.3, this substrate had a positive effect in the formation of roots and shoots in vanilla cuttings. Osorio *et al.* (2014) mention that there is a high significant interaction between the substrate and fertilization on the growth of vanilla plants, and report that when there is substrate, coconut fiber was added and produced high foliar contents of P, K, Cu, Mg and Mn, and when wood fragments were added it produced high contents of N and Ca. Some of these results agree with those found in this study and differ in others because of the components that were used to manufacture the substrates.

In foliar analyses of macro elements, it was found that the plants present a higher foliar content of potassium, followed by nitrogen and phosphorus (Table 5); these results agree with what was reported by Carrillo-González and González-Chávez (2016), who mention that K was the nutrient that accumulated most in a vanilla plant and calcium increased its concentration through time. On the other hand, Diez *et al.* (2015) report that the highest foliar content was found in Ca, K, N, and P. The nutritional demands in other species such as corn demand more nitrogen and can become a limitation in its production (Flores-Sánchez *et al.*, 2019). In stage T3 (production), there was higher foliar content of phosphorus, and in stage T1, higher amount of foliar potassium. In a study to differentiate orchids, Martínez-Monter *et al.* (2022) report values of phosphorus between 400 and 7800 ppm and of potassium from 17400 to 42600, and these ranges agree with what was found in this study.

Regarding sodium, calcium and magnesium (Table 6), a higher foliar content of calcium was found, followed by magnesium and sodium. Sodium is found in higher amount in stage T3, calcium in stage T5, and magnesium in stage T1. Vargas-Hernández *et al.* (2021) report that calcium is one of the main nutritional elements in vanilla, since it is required for the structure of cell walls and for vigor in the plant; Mg is accumulated with time, per day it accumulates 0.26 g/plant and per month 1.04 g/plant; these amounts make Mg one of the important elements because it helps photosynthesis, improves growth, and helps the health of vanilla plants.

Table 5. Total macro elements in the foliar analysis of vanilla.

Treatments	Nitrogen (%)	Phosphorus	Potassium
		ppm	
T1	1.15 a	3695.30 b	43245.70 a
T2	1.20 a	2970.70 c	35246.50 bc
T3	1.27 a	4999.50 a	31996.80 d
T4	1.14 a	3985.10 b	36496.50 b
T5	1.14 a	2680.90 c	34246.60 c
DMS	0.15	411.03	2103.40

ppm=parts per million. Values with the same letter in the lines are equal according to Tukey's test at $p \leq 0.05$. DMS=Minimum Significant Difference.

Table 6. Total micro elements in the foliar analysis of vanilla.

Treatments	Sodium	Calcium	Magnesium	Iron	Manganese	Zinc	Copper
	ppm						
T1	983.28 b	41339.50 c	3009.69 a	267.70 a	16.45 e	79.95 a	9.85 a
T2	816.59 b	36549.30 d	2018.12 e	112.75 d	29.95 d	53.45 c	9.85 a
T3	1349.87 a	29652.90 e	2428.09 b	179.95 b	33.50 c	43.20 e	6.65 b
T4	849.92 b	45541.70 b	2281.43 d	83.85 e	46.50 b	46.50 d	6.55 b
T5	816.56 b	49709.80 a	2336.43 c	130.15 c	53.50 a	59.45 b	9.85 a
DMS	193.82	947.58	27.72	5.93	0.86	0.55	0.28

ppm=parts per million. Values with the same letter in the lines are equal according to Tukey's test at $p \leq 0.05$. DMS=Minimum Significant Difference.

The micro elements were required by the plants depending on the stage of development; in the growth stage (T1), the plants presented higher foliar content of iron, copper and zinc (Table 6), before production (T2) only Cu, during production sodium, and during flowering (T5) calcium, magnesium and copper.

Osorio *et al.* (2014) report that the nutritional needs for vanilla growth under greenhouse production are 1.95% for N, 0.20% for P, 3.90% for K, 2.42% for Ca, and 1.75% for Mg. In this sense, Carrillo-González and González-Chávez (2016) found that the optimal ranges of vanilla nutrients were 1.10% to 2.43% for N, 0.16% to 2.34% for P, 2.34% to 5.64% for K, 0.48% to 2.64% for Ca, and 0.12% to 0.77% for Mg; in some of these values they agree and in others they differ from what was found in the present study because of the conditions in which the experiments were conducted.

In general, the results obtained in the substrates of this study for vanilla ought to be considered to lower the pH to slightly acidic in order to increase organic matter, which could be achieved by adding soil from the forest and pieces of wood in decomposition. On the other hand, the apparent density should be lowered, which could be achieved by adding sand or other materials such as perlite, agrolite or tezontle. Regarding the macro and micro nutrient contents found in the different substrates, it was found that all of them contain the total amounts that the plants require in their different development stages, although they should be more available for the plants to assimilate.

CONCLUSIONS

The foliar contents of macro and micro nutrients in vanilla leaves were different in the development stages of the plants, with K being the one of highest foliar content followed by N, P, Ca, and Mg. Considering these foliar contents and the total and available nutritional contents in the substrates can contribute to an adequate planning of nutrition in vanilla cultivation.

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