

Effect of Organic Substrate Composition, Fertilizer Dose, and Microbial Inoculation on Vanilla Plant Nutrient Uptake and Growth

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Keywords: biofertilization, coconut fiber, leaf litter, *Vanilla planifolia*, woodchips

Abstract

In Colombia, vanilla is a highly promising crop. The success of the plant depends, in part, on an appropriate nutrition. The nutritional requirements of the plant, however, are currently unknown. This paper seeks to answer the following questions: what is the most adequate organic substrate composition for vanilla crop? And what is the effect of chemical and biological fertilization on vanilla plant growth and nutrient uptake? Our hypothesis was that vanilla plant nutrient uptake and growth depends on the substrate composition (based on the proportion of either woodchips or coconut fiber and leaf litter), fertilization dose and biofertilization. To this purpose, experimental plots of 0.8×0.8×0.2 m were monthly filled out with 10 L of a substrate that contained either woodchips (M) or coconut fiber (F) and leaf litter (H) in different volumetric proportions (75:25, 50:50, 25:75%). Two types of fertilization were evaluated: chemical (fertilizer grade 27-11-11 at four rates: 0, 20, 60, 140 g per plant per year) and biofertilization (80 cm³ per plant of a microbial inoculum composed for several rhizosphere-microorganisms isolated from healthy vanilla plants). The results indicate that there was a significant ($P \leq 0.01$) interaction between substrate composition and fertilization on plant growth. At all dates of observation, the shoot length of vanilla plants was significantly increased with the fertilization dose of 20 g/plant only in the substrates composed by 75% of either coconut fiber or woodchips and 25% of leaf litter. In general, the presence of coconut fiber produced leaf contents of phosphorus, potassium, copper, magnesium, and manganese significantly higher ($P \leq 0.05$) than those obtained when the woodchips were included in the substrate. The magnitude of this effect depended on the nutrient considered. On the contrary, the presence of woodchips in the substrate significantly ($P \leq 0.05$) increased the leaf nitrogen and calcium contents in respect to those levels observed when the coconut fiber was employed in the growth substrate.

INTRODUCTION

Vanilla planifolia is a tropical orchid (Portéres, 1954), one of the most appreciated food flavoring world-wide used and the second most expensive spice traded in the global market after saffron (Ferrão, 1992; Ranadive, 2003; Damiron, 2004). Vanilla crop is highly promissory in Colombia given the favorable environmental conditions and the expected economical impact of the crop in some regions of the country. The crop can offer new opportunities to increase income to poor farmers as well as reduce the pressure on forests, especially in natural parks and biological reserves (Ramírez et al., 1999). However, farmers have not experience in growing vanilla in their fields.

Among the vanilla cropping demands are the kind of organic substrate and the nutrient supply. This plant grows naturally on decaying litter (Stéhlé, 1954; Anilkumar, 2004) and for its cultivation the most common substrate is leaf litter, decomposed and rotting tree bark, coconut fiber, sawdust, and vermicompost (Anilkumar, 2004; Hernandez and Lubinsky, 2010; Hernandez, 2011). However, the selection of organic material for the growth substrate seems to be critical (Ramírez, 1999; Damiron, 2004). Unfortunately, little is known about this point as well as the use of microbial inoculation that accelerate the decomposition of organic materials. Generally, vanilla crop is not fertilized beyond supplying organic substrate (Hernandez and Lubinsky, 2010). However, fertilization seems to be required because the decaying litter does not contain enough nutrients, but some authors suggested that vanilla plant are highly sensitive to high dose of fertilizers (Damiron, 2004).

The objective of this study was to evaluate the interactive effects of the type of organic materials used as substrate to roots, fertilization dose, and microbial inoculation on nutrient uptake and growth of vanilla plants.

MATERIALS AND METHODS

This study was conducted in Sopetran town (Antioquia, Colombia), which has a mean temperature of 27°C and an altitude of 1070 m. Vanilla plants used were grown in a shade-house with a light interception of 65%.

The experimental design used was split-plots, in the main were assigned substrate types and in the subplots the fertilization (chemical fertilization dose and biofertilization). Each subplot had 0.8 m x 0.8 m x 0.2 m dimensions (length x wide x depth) and in its center a plastic stick (1.2 m height) was placed as a support for shoot growth. Each subplot received bimonthly 10 L of a organic substrate composed by either coconut fiber (F) or wood chips (M) and leaf litter (H) in a proportion 75:25, 50:50, and 25:75 (V:V), respectively. The elemental compositions of these organic materials are shown in Table 1. Fertilizer (27-11-11) dose were applied at the annual rate of 0, 20, 60, and 140 g per plant. The fertilizer was applied at 90, 120, and 150 days after transplanting, close to the roots but not in direct contact to them. Microbial inoculation consisted in the application of 80 cm³ of a suspension that contained a mix of microbes (20 cm³ each) in four functional groups (nitrogen fixers, phosphate solubilizers, cellulose, and protein degraders). These microbes were previously selected from the rhizosphere of healthy three-year-old plants in vegetative stage that were growing at the same experimental site.

Treatments consisted of a 6x5 arrangement, this is, six organic substrates (F:H= 75:25, 50:50, 25:75; M:H= 75:25, 50:50, 25:75) combined with four annual dose of fertilization (0, 20, 60, and 140 g per plant) or microbial inoculation (biofertilization). Each treatment had four replications. Plants were grown for 210 days after transplanting.

Shoot length was monitored as a function on time (at 90, 120, 150, and 210 days after transplanting). At harvest, the variables measured were **shoot dry weight** (60°C for five days) and **leaf nutrient content** following the standard procedures (nitrogen: Kjeldahl method; phosphorus: molybdate-blue method; calcium, magnesium, potassium, and micronutrients: atomic absorption) carried out at the lab of biogeochemistry of the Universidad Nacional de Colombia at Medellin.

Data were subjected to analysis of variance (F-test) and LSD test (t-test) for mean separation. In both cases a level of significance ($P \leq 0.05$) was used. Statistical analyses were conducted with the software SAS System for Windows 9.0.

RESULTS AND DISCUSSION

The results showed that there were significant ($P \leq 0.05$) effects of treatments on plant nutrient uptake and growth (Table 2). Organic substrate type did not affect the shoot length and dry weight, but had significant effects on leaf nutrient contents. On the contrary, fertilization treatment significantly affected shoot length and dry weight, but it did not influence significantly the leaf nutrient content (Table 2).

A significant interaction between organic substrate type and fertilization was detected on shoot length and dry weight. At all dates of observation, the shoot length of vanilla plants was significantly increased with the fertilization dose of 20 g/plant only in the substrates composed by 75% of either coconut fiber or woodchips and 25% of leaf litter (Fig. 1). Under such conditions, at harvest the shoot length reached a peak of 229 cm when coconut fiber was included in the substrate (F75:H25), which was 41% and 27% above those found when the substrate had 50% and 25% of coconut fiber, respectively. In the case of woodchips (M75:H25) the peak was 217 cm that was 58% and 38% higher than when the woodchips had a proportion in the substrate of 50 and 25%, respectively.

On the other hand, shoot dry weight was significantly higher when the fertilizer dose of 20 g per plant was applied in the organic substrate composed by 75% of either coconut fiber or woodchips and 25% of leaf litter (Fig. 2). The increase respect to the unfertilized treatment ranged from 95% to 105% (with coconut fiber and woodchips, respectively) (Fig. 2). With the other substrates (richer in leaf litter) there were not responses on shoot dry weight to fertilization treatments.

Likely, the high proportion of coconut fiber or woodchips in the growth substrate provide favorable conditions to the root (aeration, water retention); unfortunately, this material have a slow decaying rate and cannot provide enough nutrients. Under such conditions, the fertilization provides the nutrient required for vanilla plants. Given the sensitivity of vanilla root to high dose of fertilizers, it makes sense that the positive response was detected only with the lowest rate of the fertilizer 27-11-11.

On the other hand, when the growth substrate was richer in leaf litter the decomposition process produced an environment poorly aerated that seems to be restrictive for root functioning.

The inoculation with microorganisms did not increase significantly the plant growth and nutrient uptake of vanilla plants at any organic substrate used.

Leaf nutrient content was significantly influenced by the type of organic substrate used, but not by the fertilization dose or microbial inoculation (Table 2). For instance, leaf nitrogen (N) and calcium (Ca) contents were significantly higher ($P \leq 0.05$) when the organic substrate included woodchips than when coconut fiber was used (regardless the proportion used) (Fig. 3 and 4). By contrast, the leaf phosphorus (P), potassium (K), magnesium (Mg), copper (Cu), and manganese (Mn) contents were significantly higher ($P \leq 0.05$) when coconut fiber was used instead of woodchips (regardless the proportion used) (Fig. 3 and 4). According to the work of Cibes et al. (1947), the leaf nutrient content for N, Ca, and K ranged in satisfactory levels. However, in all cases the leaf P content was very low ($< 0.15\%$). No critical levels are available for Mg, Cu, and Mn.

The higher level of Ca in the woodchips (Table 1) and the higher level of K in the coconut fiber explained the results obtained in the leaf content of these nutrients, but not for others nutrients.

The figure 4 suggests that there was an antagonism between N and K uptake and Ca and K uptake. This is consistent with the results of leaf nutrient content reported by Cibes et al. (1947). Since the woodchips contained high levels of Ca and coconut fiber contained high levels of K, it might be recommended to include both in the organic substrate.

The fact that organic substrate had significant effects on leaf nutrient contents but not on plant growth parameters need to be explained. We speculate that in the period of growth considered (seven months after transplanting) the vanilla plants were accumulating nutrients for further growth stimulation. It is logic to expect that more time would be needed to detect effects on plant growth with these treatments.

CONCLUSIONS

Vanilla plant growth was significantly higher when the fertilization dose of 20 g/plant were added in a growth substrate composed by 75% of either coconut fiber or woodchips and 25% of leaf litter. Leaf N and Ca contents were significantly higher when woodchips were used in the substrate, while leaf P, K, Mg, Mn, and Cu were significantly higher when coconut fiber was used.

ACKNOWLEDGEMENTS

This study was co-funded by the Ministry of Agriculture and Rural Development of Colombia, the Universidad Nacional de Colombia, CORANTIOQUIA, and the company BIOANDES.

Literature cited

- Anilkumar, A.S. 2004. Vanilla cultivation, a profitable agri-based enterprise. Kerala Calling: 26-30.
- Cibes, H.R., Childers, N.F. and Loustalot, A.J. 1947. Influence of mineral deficiencies on growth and composition of vanilla vines. *Plant Physiol.* 22(3): 291–299.
- Damiron, R. 2004. La vainilla y su cultivo. Dirección General de Agricultura del Estado de Veracruz, México.
- Ferrão, J.E.M. 1992. A aventura das plantas e os descobrimentos portugueses. Comissão Nacional para a Comemoração dos Descobrimientos Portugueses, Lisboa, Portugal.
- Hernandez, J. and Lubinsky, P. 2010. Cultivation systems. p. 75-95. In: E. Odoux and M. Grisoni (eds.), *Vanilla*, CRC Press Taylor & Francis Group.
- Hernandez, J. 2011. Mexican Vanilla Production. p. 3-25. In: D. Havkin-Frenkel and F.C. Belanger (eds.), *Handbook of vanilla science and technology*, Blackwell Publishing.
- Porteres, R. 1954. Le genre *Vanilla* et ses espèces. p. 24-290. In: G. Bouriquet (ed.), *Le Vanillier et la Vanille dans le Monde*, Encyclopédie Biologique, P. Lchevalier, Paris
- Ramirez, C., Rapidel B. and Matthey J. 1999. Principales factores agronómicos restrictivos en el cultivo de vainilla y su alivio en la zona de Quepos, Costa Rica. XI Congreso Agronómico Nacional y de Recursos Naturales. San Jose, Costa Rica 19-23 Jul. p. 309-313.
- Ranadive, A.S. 2003. Vanilla cultivation. *Vanilla 1st International Congress*. Princeton, New Jersey 11-12 Nov. p. 25-32.
- Stéhle, H. 1954. Ecologie. p. 291-334. In: G. Bouriquet (ed.), *Le Vanillier et la Vanille dans le Monde*, Encyclopédie Biologique, P. Lchevalier, Paris.

Tables

Table 1. Elemental composition of organic materials used in the root growth substrates for vanilla plants.

| Element | Coconut fiber (F) | Woodchips (M) | Leaf litter (H) |
|--------------------------|-------------------|---------------|-----------------|
| N (g kg ⁻¹) | 49 | 51 | 129 |
| P (g kg ⁻¹) | 10 | 9 | 11 |
| S (g kg ⁻¹) | 4 | 6 | 11 |
| Ca (g kg ⁻¹) | 19 | 123 | 218 |
| Mg (g kg ⁻¹) | 14 | 13 | 37 |
| K (g kg ⁻¹) | 160 | 30 | 40 |
| Fe (μg g ⁻¹) | 712 | 502 | 770 |
| Mn (μg g ⁻¹) | 19 | 163 | 459 |
| Cu (μg g ⁻¹) | 5 | 12 | 16 |
| Zn (μg g ⁻¹) | 12 | 22 | 48 |
| B (μg g ⁻¹) | 22 | 20 | 26 |

Table 2. Levels of significance (*P*) of the ANOVA'S (ns= non significant).

| | Organic Substrate (A) | Fertilization (B) | Interaction (AxB) |
|--------------------|--------------------------|----------------------|----------------------|
| Shoot length (90) | Ns | <0.05 | <0.001 |
| Shoot length (120) | Ns | <0.01 | <0.001 |
| Shoot length (150) | Ns | <0.001 | <0.001 |
| Shoot length (210) | Ns | <0.001 | <0.001 |
| Shoot dry weight | Ns | <0.001 | <0.01 |
| Leaf N content | <0.01 | ns | ns |
| Leaf P content | <0.01 | ns | ns |
| Leaf K content | <0.01 | ns | ns |
| Leaf Ca content | <0.01 | ns | ns |
| Leaf Mg content | <0.05 | ns | ns |
| Leaf Mn content | <0.01 | ns | ns |
| Leaf Cu content | <0.01 | ns | <0.01 |
| Leaf Zn content | Ns | ns | ns |
| Leaf Fe content | Ns | ns | ns |

Figures

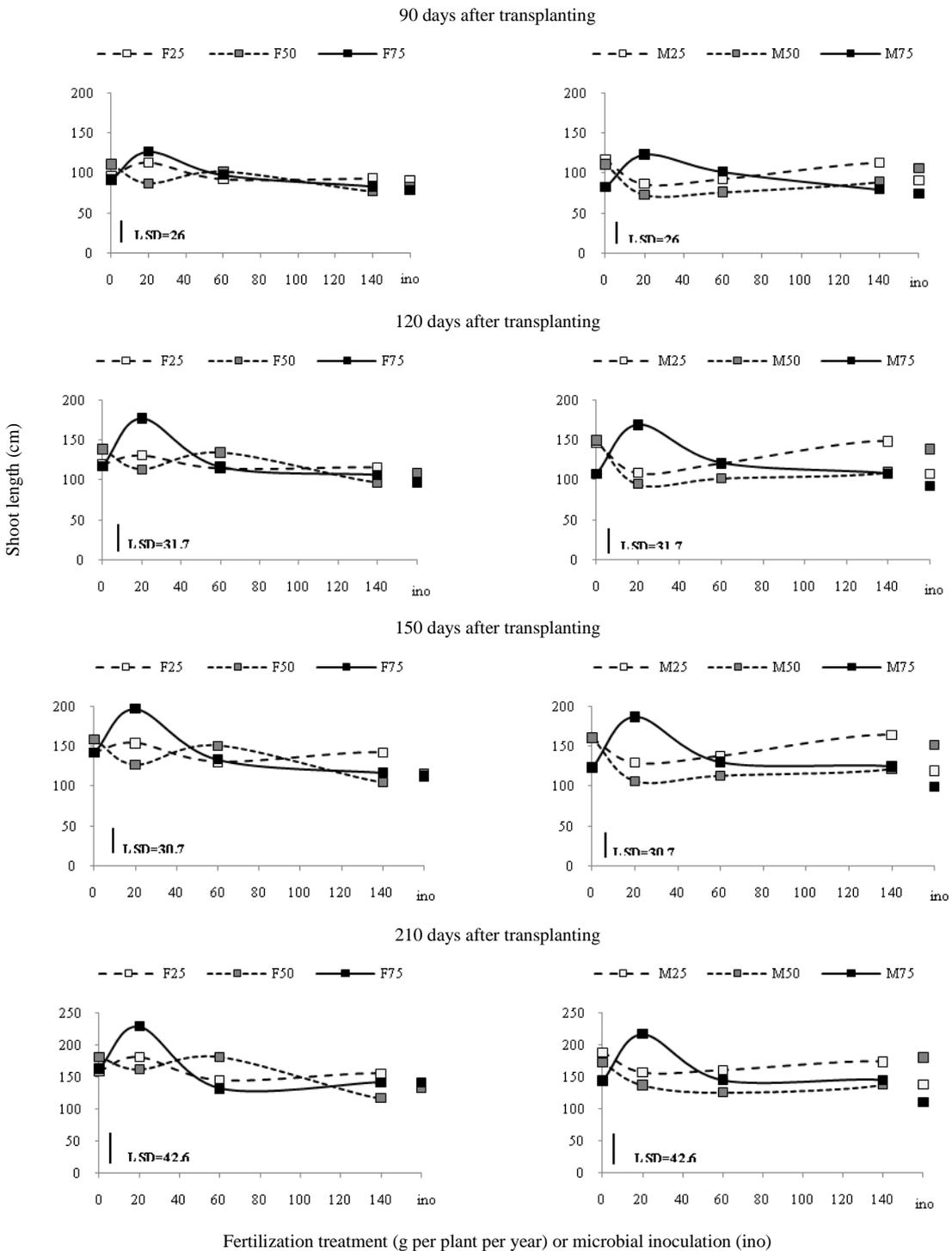


Fig. 1. Shoot length of vanilla plants as a function of treatments (fertilizer dose, microbial inoculation, and six organic substrates) at four different dates respect to transplanting. Legend numbers indicate the proportion of either coconut fiber (F) or woodchips (M) respect to leaf litter in the organic substrate. Each value is the mean of four replications. Bars represent the value of LSD ($P \leq 0.05$) in each date.

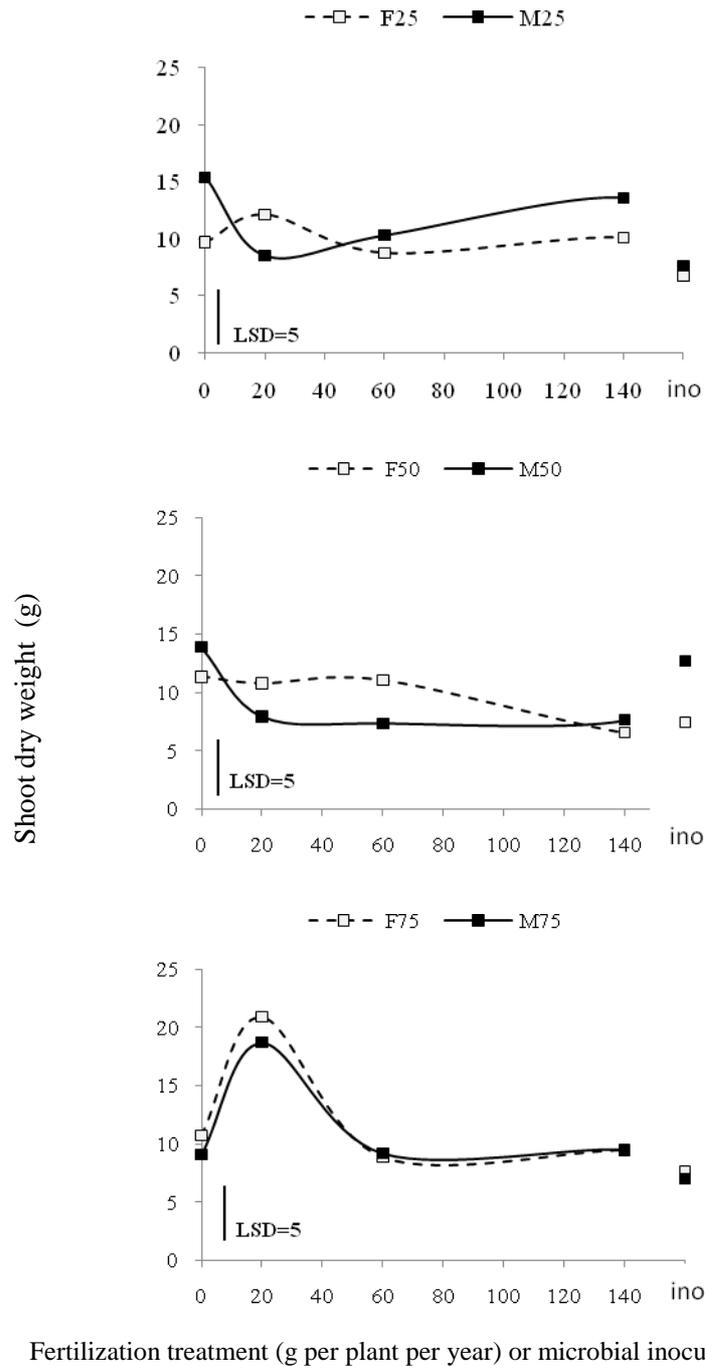


Fig. 2. Shoot dry weight of vanilla plants as a function of treatments (fertilizer dose, microbial inoculation –ino–, and six organic substrates) at harvest. Legend numbers indicate the proportion of either coconut fiber (F) or woodchips (M) respect to leaves in the organic substrate. Each value is the mean of four replications. Bars represent the value of LSD ($P \leq 0.05$) in each date.

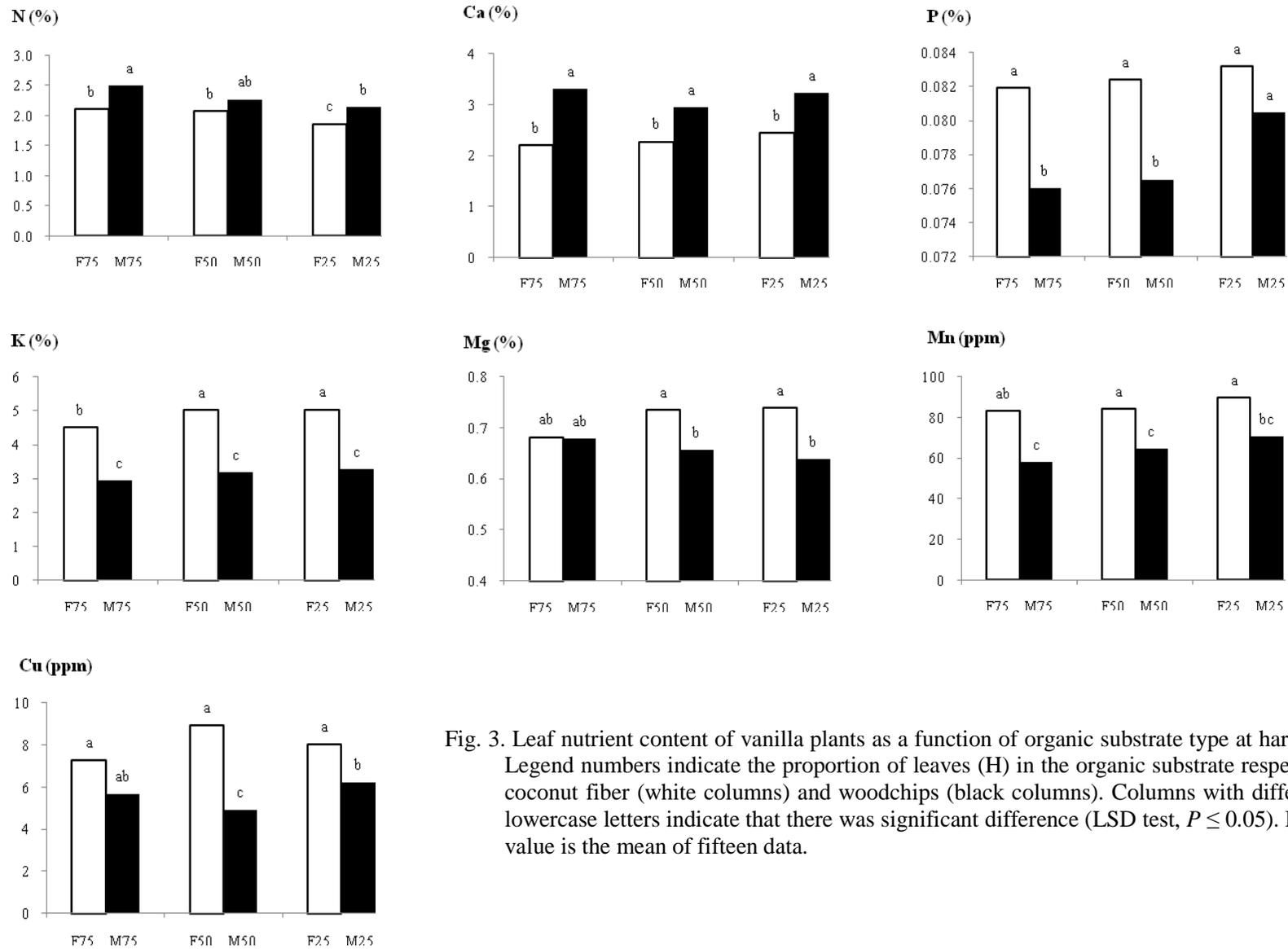


Fig. 3. Leaf nutrient content of vanilla plants as a function of organic substrate type at harvest. Legend numbers indicate the proportion of leaves (H) in the organic substrate respect to coconut fiber (white columns) and woodchips (black columns). Columns with different lowercase letters indicate that there was significant difference (LSD test, $P \leq 0.05$). Each value is the mean of fifteen data.

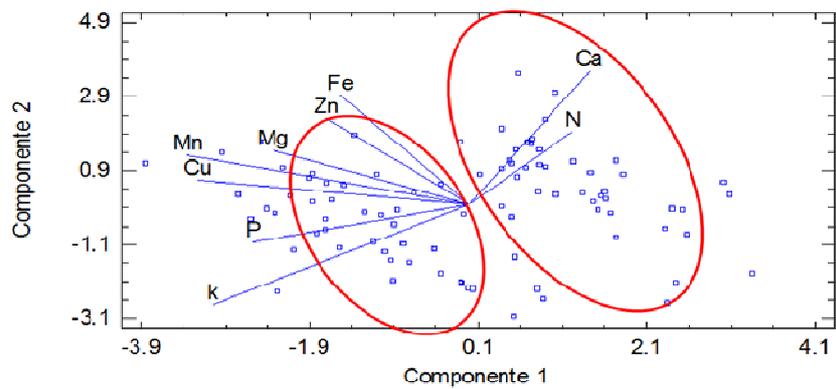


Fig. 4. Biplot of nutrient contents of leaves of vanilla plants under study. On the right side are concentrated the date for woodchips and on the left side the data for coconut fiber.