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Ammonium and nitrate ratios in the cultivation of tobacco plants

Victor Gabriel Souza de Almeida[1], Girlene Santos de Souza[2], Gildeon Santos Brito[3], Alfredo Teles de Jesus Neto[4], Cristian Martins de Souza[5], Anacleto Ranulfo dos Santos[6]

[1] Universidade Federal do Recôncavo da Bahia - UFRB, victor.gsa11@gmail.com
[2] Universidade Federal do Recôncavo da Bahia - UFRB, girlene@ufrb.edu.br
[3] Universidade Estadual Paulis - UNESP – Jaboticabal gildeon1997@outlook.com
[4] Universidade Federal do Recôncavo da Bahia – UFRB, alfredotelles008@gmail.com
[5] Universidade Federal do Recôncavo da Bahia - UFRB, cr-tiam@hotmail.com
[6] Universidade Federal do Recôncavo da Bahia - UFRB, anacleto@ufrb.edubr

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Abstract

Tobacco (*Nicotiana tabacum* L.) is a plant of the *Solanaceae* family and native to the Americas. Brazil is one of the largest tobacco producers and exporters worldwide, and this crop guarantees the income of many small rural producers. Tobacco is very demanding of nitrogen, and adequate levels of this nutrient guarantee longevity of the production chain, maximizing productivity and ensuring quality. Therefore, this study aimed to analyze the effect of ammoniacal (NH_4^+) and nitric (NO_3^-) nitrogen in the growth and development of tobacco. A randomized design was applied, using five ammonium and nitrate ratios (NH_4^+:NO_3^-): 100:0; 75:25; 50:50; 25:75 and 0:100, with five replications. After 60 days of weekly application of the solution, physiological and growth parameters were assessed: chlorophyll contents *a* and *b*; number of leaves; plant height; stem diameter; root, stem, leaf, shoot and total dry mass. The 25:75 ratio (NH_4^+:NO_3^-) resulted in an increase of height, number of leaves, stem diameter and leaf dry mass, the latter being the variable of greatest commercial relevance for tobacco cultivation. The highest chlorophyll contents were found under the 100:00 ratio (NH_4^+:NO_3^-) supplementation; however, this treatment had the lowest expressions in all other variables and is not recommended for tobacco cultivation.

Keywords: Growth. Phytomass. Ionic interaction. *Nicotiana tabacum* L. Nitrogen.

Introduction

Tobacco is an agricultural product processed from the leaves of *Nicotiana* plants, whose commercial representative is *Nicotiana tabacum* L., native to America (mainly Mexico and the Bolivian Andes), from which nicotine is extracted (AFUBRA 2022). It is an annual, biannual or perennial herbaceous plant with a single, erect stem about 2 m high, from which 18 to 26 large leaves grow, ranging 30–40 cm long and 10–20 cm wide (BARBIERI, STUMPF, 2012).

The tobacco plant has always been used by Indigenous people and traditional communities as an herbal medicine, being chewed, snuffed, inhaled in pipes or cigarettes, drunk or applied to the body (VIOTTI, 2020).

Brazil is the second largest producer of leaf tobacco, second only China. However, it is the largest exporter of this product in the world market (SOARES et al., 2023). In 2021, about 86 % of production was exported to the European Union, Asia and the United States, making tobacco one of the most relevant agricultural products for Brazil's trade balance. Data from the same year revealed that Brazilian exports amounted to 464,430 tons, generating revenue of around R$ 7.93 billion (AFUBRA, 2022).

In Southern Brazil, tobacco is grown to manufacture cigarettes; in the northeast, it is destined for the cigar industry, due to the dark tobacco used in the preparation of cigars produced in Bahia (JESUS, 2007). In Recôncavo Baiano, tobacco production dates back to the mid-17th century (LOPES, 2004), with Bahia being the cradle of tobacco cultivation in Brazil and cigars being produced in the region since the 19th century (JESUS, 2007).

Due to its social impact, tobacco cultivation is now considered a factor of human development and maintenance of people in the countryside, involving about 100,000 people who are directly and indirectly linked to the activity in the state of Bahia (OLIVEIRA, 2006). The reasonable financial return, lack of other production alternatives, even in small properties, and rational land management are pointed out as the main reasons why tobacco producers keep cultivating, despite the various economic crises the country has endure in recent decades (JESUS, 2007).

Tobacco is a crop that demands nitrogen and potassium, and balanced fertilization is necessary to replace these elements in the soil to maximize productivity and ensure quality (SOARES et al., 2023).

Nitrogen fertilization is one of the agricultural techniques that affects crop production the most (STEFEN et al., 2016). Therefore, choosing a nitrogen source is an essential decision, being a broadly researched topic, since plants show different results when exposed to different nitrogen sources (MARTINEZ-ANDÚJAR et al., 2013). However, it is necessary to know which nitrogen source is most recommended for tobacco cultivation. Moreover, nitrogen is one of the limiting chemical elements of phytomass production in natural ecosystems. Chemical forms of mineral nitrogen that plants can absorb are ammonium (NH_4^+) and nitrate (NO_3^-) ions; knowing the preference and appropriate doses of these ions for each species is essential to ensure successful agricultural production (BRITO et al*.,* 2023).

Tobacco crops should not be underdosed with nitrogen, as this leads to decreased production and leaf quality and yield. An overdose should also be avoided, since it can impact productivity, increase deficiencies and excesses on fertilization, lead to loss of income and cause environmental problems (SOARES et al., 2023).

In most agricultural crops, the absorption of only one nitrogen source can damage cell metabolism, since the excess of nitrate (NO_3^-) requires higher expenditure of cellular energy and enzymes to reduce ammonium (NH_4^+). However, ammonium does not require high energy expenditure for absorption, but high concentrations are toxic to cells, affecting plant metabolism and morphology (TAIZ et al., 2021).

The literature presents significant results when evaluating the influence of ammonium and nitrate on the Solanaceae family. For example, Tanan et al. (2019), showed the effects of nitrogen sources on fruit characteristics and physiological quality of *Physalis angulata* seeds, in which balanced ammonium and nitrate concentrations in the nutrient solution were recommended for cultivation, as they promoted larger and sweeter fruits with vigorous seeds.

Therefore, this study aimed to evaluate the initial growth of tobacco plants subjected to different ammonium and nitrate ratios as a source of nitrogen fertilization via nutrient solution.

Material and methods

This work was developed in a greenhouse on the experimental campus of the Center for Environmental and Biological Agrarian Sciences (CCAAB) of the Federal University of Recôncavo da Bahia (UFRB), in the municipality of Cruz das Almas, located in the Recôncavo Baiano, 140 km from Salvador, from March to May 2023.

Tobacco seedlings were grown from seeds acquired in local shops, using polyethylene trays with red-yellow oxisol + earthworm humus in a 2:1 ratio as substrate. The trays were kept in a greenhouse with daily irrigation, according to the climatic requirements of the crop, until transplanting, 28 days after sowing. All water used for irrigation came from the station network of the Bahia Water and Sanitation Company (Embasa), according to the chemical analysis presented in Table 1.

Table 1. Chemical characteristics of the water used for irrigation (Embasa Water)

pH	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
		------- Meq L^{-1}		
4.5	5.5	2.0	1.0	3.9

Method: EMBAPA, 2010. pH = hydrogen potential. Ca^{2+} = calcium concentration. Mg^{2+} = magnesium concentration. Na^{+} = sodium concentration. K^{+} = potassium concentration.

Note: The amount of nitrogen in the Embasa water was considered negligible due to its low concentration.

Source: authors (2024)

In the phenological stage of vegetative growth—V2 (plant with two leaves), the seedlings were transplanted into polyethylene pots with a capacity of 3.0 dm^3, filled with washed sand and vermiculite in a 2:1 ratio, respectively. Water was replaced daily in each experimental unit, with 200 mL of water per morning shift to keep the pots moist.

On the day after transplanting, a complete nutrient solution from Hoagland and Arnon (1950) with 50 % of the ionic concentration was applied to fix the plant to the inert substrate. The pH of the nutrient solution was adjusted to 5.6 (± 0.1) with HCl or NaOH when necessary. The salt concentrations of the solutions were below 1 atm for all treatments, based on the calculation of the osmotic pressure of the solution.

After one week, the plants began to receive different solutions depending on the ammonium and nitrate ratios (Table 2). The amount of nutrient solution administered in each experimental unit was equivalent to the volume of water replacement. Therefore, there were a total of five applications, in which the volume used was 1 liter of nutrient solution for each treatment.

The randomized design (CRD) was used, with a spacing of approximately 30 cm between pots. The treatments consisted of five NH_4^+:NO_3^- ratios: 100:0; 75:25; 50:50; 25:75 and 0:100,

Table 2. Volume (mL) used from the stock solutions to form 1 L of modified nutrient solution, for the half-strength, normal solution and the respective treatments (NH_4^+:NO_3^- ratios).

Stock solution	**Prepared nutrient solutions (mL L^{-1}) of NH_4^+:NO_3^-**						
	Half-strength	**Normal**	**100:0**	**75:25**	**50:50**	**25:75**	**0:100**
$KH_2PO_4^-$	0.5	1	1	1	1	1	1
NH_4Cl	-	-	15	11.25	7.5	3.75	-
KCl	-	-	5	1.25	5	3.75	-
$CaCl_2$	-	-	5	5	1.25	-	-
$MgSO_4$	1	2	2	2	2	2	2
KNO_3	2.5	5	-	3.75	-	1.25	5
$Ca(NO_3)_2$	2.5	5	-	-	3.75	5	5
Iron-EDTA*	0.5	1	1	1	1	1	1
Micronutrients**	0.5	1	1	1	1	1	1

**Micronutrients solution (g/l): H_3B0_3 = 2.86; $MnCl_2.4H_2O$ = 1.81; $ZnCl_2$ = 0.10; $CuCl_2$ = 0.04; $H_2MoO_4.H_2O$ = 0.02. *Iron-EDTA solution: 26.1 g of disodium EDTA were dissolved in 286 mL of NaOH 1 N + 24.9 g of $FeSO_4.7H_2O$ and aerated for one night.

Source: Adapted from Hoagland & Arnon (1950) as shown in Table 2, with five replications, each experimental plot consisting of a useful plant, totaling 25 experimental units. Until the end of the experiment, pest and disease attacks were monitored, and the necessary phytosanitary measures were taken to ensure integrity. In addition, a response period of the last nutrient solution application was adopted, which lasted one week.

At the end, the following variables were evaluated: plant height (H), using a tape measure in millimeters, from the neck to the apex of the terminal bud, and the results were expressed in centimeters; number of leaves (NL), determined by manual counting; stem diameter (SD), measured with a digital caliper at the base of the stem, with the results expressed in millimeters; chlorophyll contents *a* (CLA), *b* (CLB), total (TCL) and chlorophyll ratio *a/b* (RAB) using ClorofiLog—Falker-CFL1030 model, with readings taken on three leaves of the middle third of each plant, between 7 and 8 a.m.

Then, the plant material (root, stem and leaf) was individually packed in Kraft paper bags, previously identified and placed in a forced air circulation oven at 65°C ± 2°C for 72 hours, until it reached a constant mass, to determine root (RDM), stem (SDM) and leaf (LDM) dry mass. The material was weighed on an analytical scale with a precision of three decimal places. The total dry mass (TDM) was calculated with the results from the phytomass.

The data were subjected to analysis of variance and, depending on the level of significance in the *F*-test for the ammonium and nitrate ratios, Tukey's test was performed to compare means between the treatments with 5 % significance, using the SISVAR software (FERREIRA, 2019).

Results and discussion

Treatments with different ammonium and nitrate ratios significantly influenced the following growth variables of tobacco plants: plant height, number of leaves, and stem diameter ($p<0.05$); chlorophyll contents *a*, *b* and total ($p<0.05$); and the production of leaf, stem, shoot, root and total dry phytomass ($p<0.05$) (Table 3). Regarding H, NL and SD, greater plant growth was observed compared to the others when subjected to 75 % NO_3^- treatment . However, nitrate omission caused lower performance in these variables, since this crop prefers nitric fertilization (Table 4).

For H, treatments with greater nitrate concentrations (75 and 100 %) provided an increase of approximately 42.62 % compared to the treatment without nitrate, which had the lowest performance. This is due to the toxic action

Table 3. Summary of the analysis of variance for variables in *Nicotiana tabacum* L. plants subjected to different ammonium and nitrate ratios. Cruz das Almas, Bahia, 2023.

Treatment	Observed *F*-test					
	Ft	H	NL	SD	CLA	CLB
	2.87	37.3*	66.2*	267.2*	109.5*	59.8*
Treat.	Observed *F*-test					
	TCL	LDM	SDM	ShDM	RDM	TDM
	38.7*	11.7*	33.2*	30.0*	4.77*	29.0*

Treat. - treatments; Ft - tabulated *F* value / critical *F*; H - plant height; NL - number of leaves; SD - stem diameter; CLA - chlorophyll *a*; CLB - chlorophyll *b*; TCL - total chlorophyll; LDM - leaf dry mass; SDM - stem dry mass; ShDM - shoot dry mass; RDM - root dry mass; TDM - total dry mass; *all significant variables at 5 %.

Source: authors (2024)

Table 4. Plant height (H), number of leaves (NL) and stem diameter (SD) of *Nicotiana tabacum* L. plants subjected to different ammonium and nitrate ratios. Cruz das Almas, Bahia, 2023.

TREATMENT (NH_4^+:NO_3^-)	HEIGHT (cm)	NUMBER OF LEAVES	STEM DIAMETER (mm)
100:0	60.40 C	10.80 D	0.51 E
75:25	72.80 B	12.20 C	0.57 D
50:50	79.48 AB	12.40 C	0.69 C
25:75	86.14 A	16.40 A	0.91 A
0:100	81.14 A	14.40 B	0.78 B
CV (%)	4.79	4.53	3.13
Overall Mean	75.99	13.24	0.69
$\sigma \bar{x}$	1.62	0.26	0.00
$LSD_{5\,\%}$	6.88	1.13	0.04

*Means followed by the same letter in the column do not differ statistically from each other by Tukey's test at 5 % significance.

Source: authors (2023)

of ammonium when supplied in isolation, since its absorption causes electrostatic imbalance between the extra and intracellular environment, causing the cells to perform the opposite flow of positive charges to achieve neutrality (BRITO et al., 2023), leading to energy loss for charge exchange, instead of using metabolic energy for vegetative growth.

When evaluating the growth of young *Eucalyptus urophylla* plants in hydroponic medium with different ammonium and nitrate ratios, Cairo et al. (2021) obtained results in which responses of higher and lower H were significant in the 25:75 and 100:0 (NH_4^+:NO_3^-) treatments, respectively.

Regarding NL, the treatment with the best performance (75 % of NO_3) obtained a 51.85 % increase compared to plants cultivated with nitrate omission (Table 4). High concentrations of ammonium can induce morphological and physiological changes in plants. Since it does not accumulate in cell vacuoles like nitrate, ammonium becomes toxic and diminishes plant growth (MELO et al., 2020), as seen in the NL decrease.

When evaluating mint plants under different nitrate and ammonium ratios in light environments, Silva et al. (2021) obtained results similar to our study, showing that NL in plants grown in full sun under the influence of the 50:50 and 25:75 treatments (NH_4^+:NO_3^-) reached 398.5 and 333.4 leaves per plant, respectively. These treatments had the highest performances.

Regarding SD, tobacco plants cultivated with high NO_3^- concentration (75 %) associated with low NH_4^+ concentration (25 %) obtained a mean increase of 78.43 % in stem thickness compared to the treatment with no nitrate. This is due to the correct nitrogen source, enabling greater fiber constitution and, consequently, a larger SD (CAMPOS et al., 2017). Most vascular plants show severe toxic symptoms when grown only with ammonia nitrogen (BRITO et al., 2023), as evidenced in this study.

Significant responses were observed for the CLA, CLB and TCL contents for treatments with different ammonium and nitrate ratios ($p<0.05$). The 100:0 treatment (NH_4^+:NO_3^-), which provided only ammoniacal nitrogen, resulted in the highest CLA, CLB and TCL contents, while concentrations above 50 % of nitrate reduced them (Table 5).

When evaluating growth and development of *Clitoria ternatea* plants grown in different ammonium and nitrate ratios, Almeida et al. (2024) obtained results similar to ours, finding that the highest CLA, CLB and TCL contents did not necessarily correspond to higher yields, as ammonium and nitrate can reduce photosynthetic rate, stomatal opening, and CO_2 concentration in the leaves when supplied in isolation, reducing biomass production. In the case of *Clitoria ternatea*, nitrate was more toxic to the crop, while in this study, it was ammonium.

CLA and CLB are the most abundant pigments, especially CLA, used by the plant in the photochemical phase to produce chemical energy in the form of ATP and NADPH. However, CLB is an accessory pigment, helping the absorption of light in the transfer of radiant energy to the reaction centers, along with other accessory pigments (TAIZ et al., 2021). Thus, they are related to the photosynthetic efficiency of plants and, consequently, to their growth and adaptation to different environments. They are also constantly synthesized and destroyed in processes influenced by factors that are internal and external to plants (KURTZ et al., 2022).

Significant responses ($p<0.05$) were found for the phytomass accumulation variables (LDM, SDM, RDM and TDM) in response to treatments with different ammonium and nitrate ratios.

Thus, the 25:75 ratio (NH_4^+:NO_3^-) resulted in a higher mean for LDM, which is one of the most important variables considering its commercial value (Table 6). Treatments with higher concentrations of nitrate 75 and 100 % provided high averages in the production of dry phytomass, and the treatment that provided only ammoniacal nitrate showed the lowest results related to these variables.

This can be explained since most crops maximized growth with a mixed supply of ammonium and nitrate, in which some species have growth inhibited when the nitrogen source is exclusively ammoniacal (PASSOS et al., 2019). This occurred in this study, in which the 25:75 ratio (NH_4^+:NO_3^-) provided the highest yield, while the exclusively ammoniacal fertilization had the lowest yields.

Regarding LDM, the 25:75 treatment (NH_4^+:NO_3^-) provided a 65.08 % increase compared to nitrate omission, which had the lowest biomass production. Silva et al. (2018), when evaluating the dry matter yield of *Physalis*

Table 5. Chlorophyll contents *a* (CLA), *b* (CLB) and total (TCL) of *Nicotiana tabacum* L. plants subjected to different ammonium and nitrate ratios. Cruz das Almas, Bahia, 2023.

TREATMENT (NH_4^+:NO_3^-)	**CLA**	**CLB**	**TCL**
100:0	34.30 A	13.36 A	47.66 A
75:25	32.14 B	9.44 B	41.58 B
50:50	29.44 C	7.98 CD	37.42 C
25:75	26.34 D	6.62 D	32.96 D
0:100	28.66 C	8.18 BC	33.78 CD
CV (%)	2.19	8.16	5.63
Overall Mean	30.17	9.11	38.68
$\sigma \bar{x}$	0.29	0.33	0.97
$LSD_{5\%}$	1.25	1.40	4.12

*Means followed by the same letter and number in the column do not differ statistically from each other by Tukey's test at 5 % significance.

Source: authors (2023)

Table 6. Leaf (LDM), stem (SDM), root (RDM) and total (TDM) dry mass of *Nicotiana tabacum* L. plants subjected to different ammonium and nitrate ratios. Cruz das Almas, Bahia, 2023.

TREATMENTS (NH_4^+:NO_3^-)	**LDM (g)**	**SDM (g)**	**ShDM (g)**	**RDM (g)**	**TDM (g)**
100:0	5.70 B	4.37 C	10.07 B	3.01 B	13.09 C
75:25	8.12 A	7.28 B	15.40 A	3.03 B	18.43 B
50:50	9.17 A	7.45 B	16.62 A	4.28 AB	20.90 AB
25:75	9.41 A	8.40 AB	17.81 A	3.83 AB	21.65 A
0:100	8.51 A	8.91 A	17.42 A	4.72 A	22.14 A
CV (%)	11.78	9.37	8.31	20.52	8.03
Overall Mean	8.18	7.28	15.46	3.77	19.24
$\sigma \bar{x}$	0.43	0.30	0.57	0.34	0.69
$LSD_{5\,\%}$	1.82	1.29	2.43	1.46	2.92

*Means followed by the same letter and number in the column do not differ statistically from each other by Tukey's test at 5 % significance.

Source: authors (2023)

angulata L. under nitrate and ammonium ratios in hydroponic cultivation, obtained similar results, in which the 25:75 treatment (NH_4^+:NO_3^-) provided more LDM. This is because ammonium causes toxicity in plant cells, since it acts as an uncoupler between electron flow, oxidative phosphorylation, and photophosphorylation (TAIZ et al., 2021).

When evaluating SDM, supplying only NO_3^- resulted in a 103.89 % increase in dry phytomass compared to the treatment with nitrate omission; the latter had the lowest yield (Table 6). SDM is an important variable to study, since a thicker stem reflects higher productivity, greater number of leaves, and avoids possible damping-off.

Similar results were observed in the study by Lima et al. (2018) when evaluating ammonium and nitrate ratios in the growth of *Lippia alba* plants cultivated under light conditions, in which the highest SDM occurred when there was a 100 % NO_3^- supply, with a 42.67 % increase compared to nitrate omission, as occurred in this study.

Regarding ShDM, the 25:75 treatment (NH_4^+:NO_3^-) had a 76.86 % increase compared to nitrate omission, which had the lowest phytomass production and was the only treatment that differed significantly compared to the others (Table 6). By modifying nitrate and ammonium contents in nutrient solution for maize growth, Oliveira et al. (2019) obtained similar results, in which the 2.5:0.5 ratio of NO_3^-:NH_4^+ obtained a higher production of ShDM.

For RDM, supplying exclusively with NO_3^- resulted in a 56.81 % increase in dry biomass compared to the treatment with nitrate omission, which was lower than the other treatments. Our study is in line with Silva et al. (2018), with the treatment of higher production of RDM, 0:100 (NH_4^+:NO_3^-), with a 120.89 % increase compared to the 25:75 treatment (NH_4^+:IN_3^-) that made the lowest yield in this variable.

Regarding TDM, plants grown exclusively with nitrate provided a 69.13 % increase in biomass production compared to the treatment with nitrate omission, showing the lowest result. According to Lima et al. (2018), the highest TDM occurred with a 100 % NO_3^- supply, in which there was a 44.53 % increase compared to treatment with nitrate omission, as occurred in our study.

Conclusions

The 100:0 treatment (NH_4^+:NO_3^-), which exclusively provided ammoniacal nitrate, provided satisfactory increases in all chlorophyll contents; however, this treatment had the lowest performance in H, NL, SD and dry phytomass production.

Solutions with ammonium concentrations above 50 % are not recommended for this crop, since there is a decrease in productivity.

The 25:75 treatment (NH_4^+:NO_3^-) provides the best benefits for tobacco cultivation, allowing greater growth in H, NL, SD and LDM. The latter has the greatest commercial value for producers.

Treatments with higher 25:75 and 0:100 nitrate concentrations (NH_4^+:NO_3^-) are the most recommended for tobacco cultivation, as they favor better development and productivity results in NL and dry phytomass, which are commercially important variables.

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