# **Yield and composition of rosemary essential oil cultivated with different doses of nitrogen and sulfur**

Aline dos Anjos Souza1\*®, Anacleto Ranulfo dos Santos<sup>2</sup>®, Girlene Santos de Souza<sup>2</sup>®, Péricles Barreto Alvesª®, Uasley Caldas de Oliveira<sup>4</sup><sup>D</sup>, Aglair Cardoso Alves<sup>5</sup><sup>D</sup>

> Univerdidade Estadual de Santa Cruz, Ilhéus-BA, Brasil Universidade Federal do Recôncavo da Bahia, Cruz das Almas-BA, Brasil Universidade Federal de Sergipe, Aracaju-SE, Brasil Universidade Estadual de Feira de Santana, Feira de Santana-BA, Brasil Universidade Estadual do Piauí, Teresina-PI, Brasil \*Corresponding author, e-mail: eng.alinesouza@gmail.com

#### **Abstract**

Essential oils have great commercial importance for the pharmaceutical, cosmetic and food industries. Because they have therapeutic, flavoring and antioxidant properties, due to their active principles. Studies on the form of cultivation, as well as fertilization management are necessary, since information related to the production of secondary metabolites is still insufficient. Thus, the objective of this work was to evaluate the yield, content and composition of rosemary essential oil cultivated with different concentrations of nitrogen and sulfur in the soil. The treatments were distributed in a 4x2 factorial scheme with four nitrogen doses: 105, 210, 315 and 420 mg dm3, combined with two sulfur doses: 32 and 64 mg dm3 and with seven replications, totaling 56 experimental units. According to the results obtained, the combination of doses 420 of N and 64 S provided the best contents and yield of rosemary essential oil. α-pinene and 1,8-cineole had the highest concentrations for all treatments studied, with about 85% to 95% of the total constituents of the oils.

**Keywords:** medicinal plants, plant production, secondary metabolites

#### **Introduction**

Originating in the Mediterranean region, *Rosmarinus officinalis* L., popularly known as rosemary, is a species of the Lamiaceae family, with botanical synonymy *R. latifolius Mill* (Ferrari et al., 2011). It has aromatic effect, as well as use for culinary and medicinal purposes. Therefore, the economic value comes from the leaves and flowers, attributing strong camphoraceous odor and clearly fresh and spicy flavor (Prins et al., 2008).

The essential oil is used in the cosmetics and perfumery industry (Souza et al., 2014). It´s a secondary compound existing in medicinal plants that can perform important functions in the plant-pathogen interaction, through direct antimicrobial action or by potentiating defense mechanisms of other plants that may be treated with these compounds (Bonaldo et al., 2004; Hillen et al., 2012).

According to Silva et al., (2014) the essential

oil of rosemary presents in its constitution monoterpene hydrocarbons, terpene esters, linalool, verbinol, terpineol, 3-octanone and isobornyl acetate. Terpenoids are represented by carnosol, carnosylic acids, olean, ursolic, among others.

These compounds give rosemary antioxidant, antiinflammatory and antidepressant medicinal properties beneficial to health, being used to cure intercostal neuralgia, headaches, migraine, insomnia, emotional disorders and depression in popular medicine (Guo et al., 2018; Ghasemzadeh Rahbardar & Hosseinzadeh, 2020; Dabaghzadeh et al., 2022).

When analyzing the chemopreventive capacity of carnosol, one of the compounds present in rosemary essential oil, they observed its effectiveness in preventing lung and breast cancer, as well as in cutaneous papilloma. (Chun et al., 2014).

They can present several combinations of

chemical constituents, which gives them the ability to control the oxidation of food and bacteria that express high resistance to antimicrobial agents such as: *Salmonella Cholerasuis*, *Listeria monocytogenes*, *Staphylococcus aureus* and *Escherichia coli*.

Rosemary in the control of phytopathogens such as *Pseudocercospora vitis* and *Plasmopara viticola* in vine (Maia et al., 2014), *Macrophomina phaseolina* in soybean (Lorenzetti et al., 2018), *Alternaria solani, Xanthomonas vesicatoria* (ASSI et al., 2018) and *Cladosporium fulvum in tomato plants* (Itako et al., 2009), *Ralstonia solanacearum* in bell peppers (Martins et al., 2010), *Meloidogyne javanica* (Mattei et al., 2014)*, M. incógnita* in soybeans (Müller et al., 2016) *and in tomatoes (Schons et al., 2022).* 

Medicinal plants, like any other crop, require several cultivation techniques in order to determine methods that provide, simultaneously, greater accumulations of phytomass and chemical constituents of interest (Ehlert et al., 2013).

Mineral nutrients are essential for the growth and development of plants. Among these nutrients, nitrogen plays a key role in many physiological processes, especially in the production of biomass (Anas et al., 2020). It also acts in the formation of chlorophyll, proteins and proteins and other essential compounds such as plant hormones (Tiong et al., 2021). However, the excessive use of nitrogen fertilizers (N) brings undesirable consequences to the environment such as soil acidification (Mohanty et al., 2020), so adopting strategies for better use and utilization are necessary.

Having said that, it´s known that sulfur is a fundamental element necessary for nitrogen metabolism and protein synthesis, which is mandatory for many enzymatic and metabolic activities, encompassing the plant's defense systems against biotic or abiotic stresses (Khan et al., 2014; Yu et al., 2018; Tavanti et al., 2021).

Ngezimana (2013) states that nitrogen and sulfur have a direct relationship, so if sulfur deficiency occurs, nitrogen absorption will significantly decrease. Therefore, the application of N and S needs to be balanced for better yields. Amanullah et al., (2011) when evaluating the effect of nitrogen and sulfur on the production of canola oil, they reached the highest concentration of oil at the dose of 60 Kg ha<sup>-1</sup> of sulfur together with 80 Kg ha<sup>-1</sup> of nitrogen. Studying growth and nutritional diagnosis in rosemary plants fertilized with nitrogen and sulfur Souza et al., (2020) found that the assimilation of S in the leaves was dependent on the doses of N.

Therefore, studies on the management of fertilization of the Brazilian medicinal flora make it possible

to generate ideal conditions of cultivation in order to raise satisfactory indices in the production of dry matter mass and active principles of economic interest. Therefore, the objective of this study was to evaluate different concentrations of nitrogen and sulfur in the production and composition of rosemary essential oil.

## **Materials and Methods**

The experiment was conducted in a greenhouse located on the experimental farm of the Center for Environmental and Biological Agrarian Sciences of the Federal University of Recôncavo da Bahia (*Universidade Federal do Recôncavo da Bahia*) in the county of Cruz das Almas - BA, which is geographically located at 12°40'19"S and 39°06'22"W at an altitude of 212 m. The soil used in the experiment was classified as a Distrocoeso Argisol (Ericsson & Ronge, 2006). Plastic vessels with the capacity of 3,0 dm3 were filled with the soil collected in the 0,0 to 0,20 m of depth, the analyses of chemical and textural attributes are shown in **Tables 1** and **2**, respectively:

0.96

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 $2.6$ 

7.32

 $\overline{\circ}$ 

 $0.0 - 20$ 

soil organic matter: V - Base saturation

B-Sum of bases; CTC(t) - Effective cation exchange capacity; CTC(T) = cation exchange capacity to pH = 7; MO

**Table 1.** Chemical attributes of the Argisol used for the cultivation of Rosemary (*Rosmarinus officinalis* L.) in depth of 0.0 – 20 m, Cruz das Almas, BA, 2019 Prof. pH P K S Ca Mg Al Na H+Al SB CTC V M.O (cm) H20 mg dm-3 ..........Cmolc dm-3 .. % g Kg 0.0 - 20 5.6 0.1 7.32 2.6 0.8 0.5 0.0 0.00 1.5 1.2 2.82 47 0.96 SB - Sum of bases; CTC(t) - Effective cation exchange capacity; CTC(T) = cation exchange capacity to pH = 7; MO - soil organic matter; V - Base saturation.able 1. Chemical attributes of the Argisol used for the cultivation of Rosemary (Rosmarinus officinalis L.) in  $\frac{0}{5}$ % g Kg **CTC** 9  $rac{1}{2}$  $\frac{8}{2}$  $dm<sup>3</sup>$ .Cmol.  $\overline{\mathbf{z}}$  $\frac{9}{5}$ 8 2019 BA, Cruz das Almas, ma dm<sup>-3</sup>  $\frac{1}{2}$ depth of  $0.0 - 20$  m, 공  $(cm)$ Prof.





The experimental design was completely randomized in a 4x2 factorial scheme, with four nitrogen rates: 105, 210, 315 and 420 mg dm<sup>3</sup> of soil and two doses of sulfur: 32 and 64 mg dm<sup>3</sup>, with seven replications totaling 56 experimental units.

The following doses of micronutrients were applied to each vessel: boron (B) = 0.81 mg dm $^3$ of H $_{\rm_3}$ BO $_{\rm_3}$ copper (Cu) =  $1.33$  mg dm<sup>-3</sup> of CuSO<sub>4</sub>.5H<sub>2</sub>O; molybdenum  $(Mo) = 0.15$  mg dm<sup>3</sup> of  $(NH_4)$  6Mo7O<sub>24</sub>.4H<sub>2</sub>O; manganese  $(Mn) = 3,66$  mg dm<sup>3</sup> of MnCl<sub>2</sub>.H<sub>2</sub>O and zinc (Zn) = 4,0 mg dm $^3$  of ZnSO<sub>4</sub>.7H<sub>2</sub>O as indicated by Alvarez (1974). The concentration of each nutrient was provided via complete nutrient solutions modified according to Hoagland & Arnon (1950) (**Table 3**).

The rosemary seedlings were obtained by cuttings from mother plants and produced in polyethylene plastic bags with 1,0 dm3 of substrate (70% soil + 30% worm humus). The best seedlings were selected and transplanted into the pots, remaining for a period of 120 days.

 Leaves were collected, individually packed in paper bags, taken to an oven with forced air circulation, 45 ºC, for 96 hours. Subsequently, they were weighed to obtain the dry mass (grams) and destined for the extraction of essential oil through the method of hydrodistillation by water vapor drag, equipped with a graduated Clevenger type apparatus (Santos et al., 2004 a). 1 g of dry mass was used to determine the moisture content, and the samples were dehydrated at a temperature of 70º C, up to constant weight. It was necessary to join the dry material of the repetitions of each treatment, to obtain the amount of phytomass sufficient for oil extraction, according to Santos et al., (2004 a). We used 20 g of each sample, placed in a 1 L glass flask containing distilled water in sufficient volume for total coverage of the plant material. Graduated Clevenger devices were used, coupled to glass balloons, heated by electric thermal blankets with thermostat. The extraction process was conducted for 2 hours, counted from the condensation of the first drop of essential oil, and the volume extracted in the graduated column of the Clevenger was verified. Subsequently, with the use of the Pasteur pipette, the oil was packed in a glass bottle with a capacity of 2 mL, labeled and stored in a commercial freezer at -5ºC until the chemical analysis was performed. The extraction of the essential oil from Rosemary plants was performed in the Photochemistry Laboratory.

 The content of rosemary essential oil was obtained according to the methodology described by Santos et al., (2004a), where content calculation (Equation 1) was performed from the moisture-free base (BLU), which corresponds to the volume (mL) of essential oil in relation to dry mass.



Where: To = oil content (%); Vo = Volume of oil extracted (mL); MS = Plant phytomass (g); U (%) = Amount of moisture present in the biomass.

The oil yield was obtained by the value of the content/100 and multiplied by the mass of the total dry matter of the leaves.

The analyses of the oil extracted from the dried leaves were performed using a CG-EM/CG-DIC (GC-2010 Plus; GCMS-QP2010 Ultra, Shimadzu Corporation, Kyoto, Japão) equipped with an autosampler AOC-20i (Shimadzu). The separations were performed using a fused silica capillary column Rtx®-5MS Restek (polysiloxane 5% - diphenyl - 95% - dimethyl) of 30 m x 0,25 mm inner

Table 3. Volume (mL) of stock solutions to form 1 L of modified nutrient solution, using combinations of nitrogen and sulfur according to the respective treatments.



\*\*Micronutrient solution (g/l): H<sub>3</sub>B0<sub>3</sub> = 2,86; MnCl<sub>2</sub> 4H<sub>2</sub>0 = 1,81; ZnCl<sub>2</sub> = 0,10; CuCl<sub>2</sub> = 0,04; H2Mo0<sub>4</sub> H<sub>2</sub>0

diameter (d.i.), 0,25-µm of film thickness, in a constant flow of Helium (99,999%) with rate of 1.2 mL.min<sup>-1</sup>. Was used an injection volume of 1 µL (10 mg.mL-1), with a reason of *split* of 1:30. The temperature programming of the oven used was from 50°C (isotherm during 1,5 min), with an increase of 3 °C/min, to 240 °C, then, to 10°C / min until 300 °C, ending with a 7 min isothermal to 300°C.

The components of the oil were identified through the visual comparison of its mass spectrum with the existing spectra in the literature (Adams, 2017), as well as spectra from the database using the libraries provided by the device (WILEY8, NIST107 and NIST21). The libraries allow the comparison of the spectra of the constituents with those of the literature of the equipment using a similarity index of 80. The relative retention ratios (IRR) were determined using an homologous series of *n*-alkanes  $(C_{8}$ -C<sub>30</sub>) injected under the same chromatographic conditions as the samples, using the equation of Van Den Dool & Kratz (1963). It´s noteworthy that it was not possible to perform statistical analysis for the results of essential oil, due to the grouping of samples to obtain a greater amount of phytomass of the leaves.

#### **Results and Discussion**

Regarding the essential oil contents (**Figure 1**), the treatment 420 mg dm<sup>3</sup> N with 64 mg dm<sup>3</sup> S obtained the highest mean percentage value of 2.53%, and the lowest content of 1.59%, which corresponds to the dose of 105 mg dm<sup>3</sup> from N 32 mg dm<sup>3</sup> S, respectively.

All doses of N combined with 64 mg dm $3$  from S were responsible for the highest percentage values when compared to 32 mg dm<sup>3</sup> S. Demonstrating that the higher N and S ratios positively interfere in the essential oil contents of rosemary, also proving that the recommendation of Hoagland & Arnon (1950) of 64 mg dm $3\,$  S it's ideal for most cultures applying to rosemary.



Barros (2000) explains that in the synthesis of

**Figure 1 -** Rosemary essential oil content (*Rosmarinus officinalis* L.) grown with doses of Nitrogen and Sulfur in greenhouse. Cruz das Almas, BA, 2019.

proteins is considered on average have about 34 nitrogen atoms for each atom of sulfur. While the concentration of sulfur in proteins is 1 g kg<sup>-1</sup> and nitrogen is fifteen times higher. This explains the results found, in which the best results are attributed to the doses of N combined with the highest dose of S of 64 mg dm<sup>3.</sup>

Seixas et al., (2013) evaluating mineral fertilization (NPK) in the production, content and composition of essential oil citronella grass, found the highest contents of essential oil at doses of 100% (1.59%) and 150% of NPK (1.67%), and the recommendation of mineral fertilization was 100 kg ha<sup>-1</sup>  $P_2O_5$ , 40 hg ha<sup>-1</sup> K<sub>2</sub>O and 50 kg ha<sup>-1</sup> supplied by simple superphosphate, potassium taps and ammonium sulfate. However, in aromatic and medicinal plants, nitrogen has a controversial role in relation to the production of secondary metabolites, as he pointed out, Guerra et al., (2020) when evaluating the influence of nitrogen on biomass accumulation and basil essential oil yield (*Ocimum basilicum*), observed that there are increases in the contents of essential oil up to the dose of 150 kg ha<sup>-1</sup> in the first cut, and the of 250 kg ha<sup>-1</sup> in the second cut, and from these values reduction in the contents of essential oil, the authors attribute the reduction of the contents of essential oil, due to the luxurious increase in the accumulation of biomass in the form of structural components per unit area, because in these situations, one can have plants with larger specific leaf area and leaf area ratios, that imply thicker leaves, however, these components do not necessarily contribute to increases in essential oil contents.

Studying the biomass production, content and composition of the essential oil of *Mentha* x *piperita* L as a function of different sources and doses of nitrogen Deschamps et al., (2012) did´nt have a significant effect on essential oil content and production. They achieved the reduction by increasing the nitrogen dose of 20 for 40 kg ha<sup>-1</sup>, in the two sources tested, obtaining the highest contents (approximately 40 µL g-1 ms) with the doses of  $20$  kg ha<sup>-1</sup> nitrogen in the form of urea and 30 kg ha $^{-1}$  of nitrogen in the form of ammonium sulfate. Essential oil productivity was also reduced when urea was used, from 77.0 L ha<sup>-1</sup> to 58.9 L ha<sup>-1</sup>. In the use of ammonium sulfate increased the productivity of essential oil of 65,9 L ha-1 to 81,8 L ha-1.

 This study demonstrates similarity with the results obtained with rosemary, where the presence of sulfur provided a better efficiency in nitrogen absorption, reflecting in higher production rates, for this crop the best N:S ratios correspond when sulfur is supplied in the dose of 64 mg dm3 regardless of the dose of N used.

However, for some species the results may be contradictory. Studies such as Mapeli et al., (2005) revealed that the production of oil in floral chapters of chamomile did not suffer significant effect for fertilization with up to 60 kg ha<sup>-1</sup> of N and 200 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5.</sub>

 Regarding the essential oil yield of rosemary plants, the results were similar to those found for the content, where the highest average yield value was attributed to the dose 420 mg dm<sup>3</sup> N and 64 mg dm<sup>3</sup> S reaching  $(0.72 \text{ g kg}^{-1})$  and the lowest value of  $(0.40 \text{ g kg}^{-1})$ corresponding to the treatments of  $105$  mg dm<sup>3</sup> and  $315$ mg dm $3$  of N with 32 mg dm $3$  S respectively. Following the same behavior of the content results, the nitrogen doses combined with 64 mg dm<sup>3</sup> of S, were better, indicating a greater balance in these N:S ratios, consequently reflecting in the increase in rosemary essential oil yield (**Figure 2**).



**Figure 2 -** Rosemary essential oil yield (*Rosmarinus officinalis* L.) grown with doses of Nitrogen and Sulfur in greenhouse. Cruz das Almas, BA, 2019.

The yield of essential oil can be affected by a number of factors, one of these are the sources of fertilizers used. According to Tawfeeq et al., (2016) Studying different sources of fertilizers for rosemary, as well as its form of application stated that the volume of essential oil obtained from the plant differs, depending on the type of fertilizer used. Where organic source treatments obtained superior results than inorganic source fertilizers.

However, the addition of N in the cultivation of medicinal plants has shown importance in the increase of dry mass and in the yield of essential oil. Rao (2001), states that most aromatic plants are sensitive to N deficiency, and that adequate supply of this increases the production of essential oil.

Costa et al., (2010) that by studying different levels of nitrogen fertilization in the yield and composition of essential oil of patchouli, obtained maximum productivity of dry mass of leaves and essential oil in the application of 99 Kg ha<sup>-1</sup> of nitrogen. The results of this study corroborate

with the literature the addition of N proportionally increased the content and yield of rosemary essential oil, the results also reveal that the efficiency of N utilization is intrinsically related to the presence of S in adequate amounts in the solution.

Regarding the results of rosemary essential oil composition, it´s noticed that there are changes in the amount and types of compounds found with the variation of nitrogen and sulfur doses. For most treatments with the supply of sulfur in the dose of 64 mg dm<sup>3</sup>, a higher quantity of the compounds found showed that the N:S ratio in equilibrium is beneficial and may interfere with the quality of rosemary essential oil.

 The major compounds for all treatments were: α- pinene and the 1,8- cineole, representing about 85% to 95% of the total compounds. The highest concentrations of -pinene were found in the doses 315 mg dm3 of N with 64 mg dm3 S (65.79%) and 420 mg dm3 of N with 32 mg dm<sup>3</sup> S (64.87%), and 1.8 cineole at doses 210 mg dm<sup>3</sup> of N with 32 mg dm<sup>3</sup> of S (31.50%) and 105 mg dm<sup>3</sup> of N with 32 mg dm3 of S with the content of (31.25%). It was also observed that some compounds were detected only in some treatments, such as limonene which was detected in two treatments specifically being these 105 mg dm<sup>3</sup> of N with 64 mg dm<sup>3</sup> of S and 210 mg dm<sup>3</sup> of N with 64 mg dm<sup>3</sup> of S respectively. The same was true of the constituents cis-pinocanfona and the γ-terpineol detected exclusively at dose 210 mg dm3 of N with 64 mg dm3 of S (**Table 4**).

Twelve different compounds were found in rosemary essential oil when grown with different doses of nitrogen and sulfur (table 1), including camphene, pinene, camphor, borneol, verbenone, bonyl acetate, and an unidentified constituent.

Several factors can affect the composition of rosemary essential oil, in the literature it´s possible to find studies such as that of Kocak et al., (2021) that when evaluating the composition of rosemary essential oil, found, camphene (22.45%), 1.8-cineole (35.36%), linalool (3.67%), camphor (10.80%), cyclohexane, (1-methylethylidene) (3.09%), α-fenkyl alcohol (3.03), 2-cyclohexen-1-one, 2-methyl-5-(1-methylethenyl) (2.12%) and endobornyl acetate (4.50%) as the main compounds. By analyzing the chemical composition and antibacterial activity of rosemary essential oil from three distinct regions Outabia et al., (2016) found 1.8 cineole as the main component, pointed out that these results differ when compared to other studies, such as that of Lograda et al., (2013) that identified camphor (42.7%) as the majority constituent of rosemary essential oils, originating from different regions of Algeria.

**Table 4** - Chemical composition (%) of rosemary leaf essential oil (Rosmarinus officinalis) analyzed by CG-EM. (GC-EM: gas chromatography-mass spectrometry), Cruz das Almas, BA, 2019



Corroborating with the results found in this study, Mekonnen et al., (2016) obtained α-pinene (50.83%) as the major constituent of rosemary oil, followed by camphene (5.21%), ß-pinene (2.06%), ß-myrcene (0,68%), 1,8-cineole (24.42%), camphor (3.84%), borneol (1.51%) and acetate of borneol (1.62%), verbenone (0.52%), linalool (1.26%) and limonene (1.72%) most of these are also identified in this study.

Generally, monoterpenes are the main components of rosemary essential oil. Takayama et al., (2016) have suggested that these compounds are among those responsible for the antioxidant action of rosemary essential oil. In their studies they affirm the antioxidant activity, and that in the composition of the oil there was, 28.5% of cineole, 27.7% of camphor and 21.3% of α-pinene.

Evaluating the ethnopharmacological, phytochemical and some biological activities of rosemary and essential oil through literature review. Borges et al., (2018) found that the anti-inflammatory activity of rosemary essential oil can be conferred mainly to 1,8-cineole and α-pinene, as they are major and wellelucidated components. They also mention camphor, and report that even if its efficiency is proven in *in vitro* studies, there are unknown mechanisms that need to be clarified.

According to the results in the composition of rosemary essential oil found and compared with other studies, it's perceived that variability occurs, being an understandable fact, since this occurs due to intrinsic factors (genetics, subspecies) or extrinsic, such as climate and soil (geographical origin) or extraction method (Özcan e Chalchat, 2008).

Generally, medicinal plants when subjected to low fertility environments have a higher production of secondary metabolites, particularly phenolic derivatives, but this does not occur in conditions of low availability of nitrogen and sulfur, in which the production of secondary metabolites is decreased (GOBBO-NETO & LOPES 2007). These results justify the importance of having studied the N and S relationship in the cultivation of rosemary plants.

#### **Conclusions**

The content and yield of rosemary oil differ according to the doses of nitrogen and sulfur used.

The combination between the doses 420 mg dm<sup>3</sup> N with 64 mg dm<sup>3</sup> S was the one that provides the best content and yield of rosemary essential oil.

The α-pinene and the 1,8-cineole, are the major compounds for all doses studied, representing about 85% to 95% of the total compounds found in this study.

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